

N_2 FIXATION BY BLUE-GREEN ALGAE IN TWO SOUTH SWEDISH
LAKES

METHODOLOGY AND EFFECTS OF SEWAGE DIVERSION AND LAKE
RESTORATION

LARS LEONARDSON

INSTITUTE OF LIMNOLOGY

UNIVERSITY OF LUND, SWEDEN

LUND 1984



N₂ FIXATION BY BLUE-GREEN ALGAE IN TWO SOUTH SWEDISH LAKES

Methodology and effects of sewage diversion and lake
restoration

Lars Leonardson

CE, Ög

Akademisk avhandling som för avläggande av filosofie dok-
torsexamen vid Matematisk-naturvetenskapliga fakulteten
vid Universitetet i Lund kommer att offentligen försvaras
å Limnologiska institutionen, Fabriksgatan 2, Lund,
fredagen den 11 maj 1984, kl 10.15.

Organization LUND UNIVERSITY Institute of Limnology Box 3060 S-220 03 Lund, Sweden	Document name DOCTORAL DISSERTATION	
Author(s) Lars Leonardson	Date of issue 1984-05-11 CODEN: LUNBDS/(NBLI-1012)/1-111/(1984) Sponsoring organization National Swedish Environmental Protection Board	
Title and subtitle N ₂ fixation by blue-green algae in two South Swedish lakes Methodology and effects of sewage diversion and lake restoration		
Abstract Blue-green algal nitrogen fixation was studied in the field and laboratory using the indirect acetylene-reduction method.		
Methodological studies showed that 1. introduction of Lugol's solution as a preserving agent resulted in increased solubility of ethylene and in a reaction between the gas and fixative. By using a standardized method it is possible to correct for the loss of ethylene. 2. Concentration of algae prior to the acetylene assay resulted in varying effects on specific nitrogenase activity among lakes studied, and among sampling dates. Thus, concentration should not be done when using the acetylene method.		
Sewage-water diversion from eutrophic Lake Södra Bergundasjön resulted in a drastic change in lake N metabolism, and replacement of non-N ₂ -fixing algal species by a heterocystous blue-green alga, <i>Anabaena spiroides</i> . Before the diversion N ₂ fixation did not occur, but afterwards 60-85% of the total nitrogen input to the lake during summer was by N ₂ fixation.		
The restoration of Lake Trummen caused great changes in water and sediment chemistry, and phytoplankton assemblage composition. After restoration N ₂ fixation successively decreased because of P depletion. During the last study year, experimentally-manipulated interactions between fish, zooplankton and phytoplankton affected heterocystous algae severely, and N ₂ fixation was extremely low.		
Comparison between estimated nitrogen demand of N ₂ -fixing algae and N ₂ -fixation rates revealed incomplete nitrogen supply by N ₂ fixation, and suggested that nitrate and ammonium might be assimilated simultaneously or sequentially with N ₂ fixation in heterocystous algae.		
Key words N ₂ fixation, nitrogen fixation, nitrogenase activity, acetylene reduction, Lugol's solution, sewage diversion, lake restoration		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title 0348-0798		ISBN
Recipient's notes	Number of pages 111	Price
	Security classification	

Distribution by (name and address)

Lars Leonardson, Institute of Limnology, Box 3060, S-220 03 Lund, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Lars Leonardson

Date 1984-04-06

N_2 FIXATION BY BLUE-GREEN ALGAE IN TWO SOUTH SWEDISH LAKES

METHODOLOGY AND EFFECTS OF SEWAGE DIVERSION AND LAKE RESTORATION

LARS LEONARDSON

CE, Ög

INSTITUTE OF LIMNOLOGY

UNIVERSITY OF LUND

LUND, SWEDEN

DISSERTATION

LUND, 1984

A dissertation for the Doctor's Degree
in Natural Sciences
Institute of Limnology
University of Lund

ABSTRACT

Blue-green algal nitrogen fixation was studied in the field and laboratory using the indirect acetylene-reduction method.

Methodological studies showed that 1. introduction of Lugol's solution as a preserving agent resulted in increased solubility of ethylene and in a reaction between the gas and fixative. By using a standardized method it is possible to correct for the loss of ethylene. 2. Concentration of algae prior to the acetylene assay resulted in varying effects on specific nitrogenase activity among lakes studied, and among sampling dates. Thus, concentration should not be done when using the acetylene method.

Sewage-water diversion from eutrophic Lake Södra Bergundasjön resulted in a drastic change in lake N metabolism, and replacement of non-N₂-fixing algal species by a heterocystous blue-green alga, Anabaena spiroides. Before the diversion N₂ fixation did not occur, but afterwards 60-85% of the total nitrogen input to the lake during summer was by N₂ fixation.

The restoration of Lake Trummen caused great changes in water and sediment chemistry, and phytoplankton assemblage composition. After restoration N₂ fixation successively decreased because of P depletion. During the last study year, experimentally-manipulated interactions between fish, zooplankton and phytoplankton affected heterocystous algae severely, and N₂ fixation was extremely low.

Comparison between estimated nitrogen demand of N₂-fixing algae and N₂-fixation rates revealed incomplete nitrogen supply by N₂ fixation, and suggested that nitrate and ammonium might be assimilated simultaneously or sequentially with N₂ fixation in heterocystous algae.

CONTENTS

LIST OF PAPERS	4
INTRODUCTION	5
METHODOLOGICAL ASPECTS	6
ECOLOGY OF N_2 -FIXING BLUE-GREEN ALGAE	7
MANAGEMENT MEASURES AND N_2 FIXATION	8
NITROGEN BUDGETS	10
FUTURE RESEARCH	12
TACK	13
REFERENCES	13
PAPER I	
PAPER II	
PAPER III	
PAPER IV	
PAPER V	

The thesis is based on the following papers:

- I. Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water. - Can. J. Microbiol. 26:599-605.
- II. Effects of concentrating phytoplankton on the acetylene-reduction assay for nitrogenase activity.
- Freshwat. Biol. 13:265-274.
- III. Effects of sewage diversion in Lake Södra Bergundasjön.
II. Phytoplankton changes and the role of nitrogen fixation. - Verh. Internat. Verein. Limnol. 20:2701-2707.
- IV. Blue-green algal nitrogen fixation in Lake Trummen after restoration. - Manuscript.
- V. Does N_2 fixation meet the nitrogen requirements of heterocystous blue-green algae in shallow eutrophic lakes?
- Submitted to Oecologia (Berl.).

The articles are reprinted with the kind permission of Can. J. Microbiol. (I), Freshwat. Biol. (II) and Verh. Internat. Verein. Limnol. (III).

INTRODUCTION

The availability of nitrogen to organisms is ultimately regulated by the processes of N_2 fixation and denitrification cycling N between the atmosphere and biota. Postgate (1978) estimated global biological N_2 fixation to amount to c. 2×10^8 tonnes per year. Industrial production of fertilizer supplies an additional 10-20%. In many ecosystems, e.g. forests, coastal waters, and oceans, nitrogen is scarce, and nitrogen fixation and denitrification are of great importance for the growth and biomass development of populations. In freshwater lakes phosphorus is usually the ultimately limiting nutrient (Schindler 1977). However, in eutrophic and hypertrophic lakes, in which phosphorus and other nutrients, except nitrogen, are internally supplied by regeneration from nutrient-rich sediments, availability of dissolved inorganic nitrogen (DIN) is often limiting for phytoplankton production (Claesson and Ryding 1977). Under such conditions, N_2 fixation mainly mediated by heterocystous blue-green algae allows the development of high biomass and production. This in turn often results in rising pH in the water, and increased organic load at the sediment surface, which may induce further nutrient release. The dinitrogen fixed is made available to non- N_2 -fixing algae by release from decomposing dead N_2 fixers, by excretion, and by leakage from the junctions between heterocysts and vegetative cells.

Although biological nitrogen fixation has been known for about a century (Fogg et al. 1973), large-scale in-situ quantification was not possible until the end of the 1960's. Due to the discovery that nitrogenase, the N_2 -fixation enzyme, converts acetylene into ethylene under inhibition of N_2 fixation (Dilworth 1966, Schöllhorn and Burris 1966), ^{15}N methodology could be replaced by the acetylene-reduction technique. The latter method involves cheaper and more convenient procedures, as well as shorter incubation times. It is, however, an indirect

method. Nevertheless, it was widely accepted, and meant a break-through for N_2 -fixation research.

My work at the Institute of Limnology in Lund has been directed towards the study of N_2 fixation in some eutrophic South Swedish lakes. The purpose was to explore what factors regulate the process, how much N is fixed, and what is the importance of this nitrogen in comparison with other N sources. In my Ph. D. thesis I present results of methodological studies, and effects in dinitrogen fixation induced by sewage diversion and lake restoration. I also tried to estimate whether or not N_2 fixation covered the total nitrogen demand in heterocystous blue-green algae.

METHODOLOGICAL ASPECTS

A systematic study of the limitations of the acetylene-reduction method did not appear until 1974 (Flett et al. 1975). Thus, many workers, including myself, made many mistakes.

Since Stewart et al. (1967, 1971) found no negative effects of concentrating algae before the acetylene assay, most followers have used this procedure. However, I found that the concentration only rarely retained 100% of the heterocystous algae, and that the specific nitrogenase activity was sometimes much affected. Occasionally it was not affected at all. Since the effects varied between lakes, and between sampling occasions, it was concluded that samples should not be concentrated (II). Consequently, N_2 -fixation data presented during the 1970's were often underestimates. Only when verified that there was no negative concentration effect data should be relied on.

Due to destruction of cells, the use of conventional conservation agents did not allow counting of algae from the incubation vessels. Introducing Lugol's solution as fixative, I found that the solubility of ethylene in the liquid phase increased in the presence of Lugol's solution, and that there was a reaction between the fixative

and ethylene. However, the effects could be predicted and corrected for, hence the fixative could be used (I).

Stewart et al. (1967) found that the N_2 -fixation enzyme was saturated at a partial pressure of 0.1 atm C_2H_2 in the vapor phase. However, Flett et al. (1976) showed that the proportion of vapor-phase volume to gas-phase volume determines the amount of dissolved acetylene. Experimentally I found that nitrogenase activities measured in Lakes Södra Bergundasjön and Trummen were underestimated when a pC_2H_2 of 0.1 atm was used in my systems. Therefore, after 1976 I added a saturating double amount of acetylene to the algae, and rates in papers IV and V measured before 1977 were corrected by a factor 1.4. The same factor should be applied to data in paper III to give correct values.

ECOLOGY OF N_2 -FIXING BLUE-GREEN ALGAE

Fogg et al. (1973), outlining the environmental requirements for N_2 -fixing blue-green algae, stated the prerequisites of low concentrations of dissolved inorganic nitrogen (DIN), and high availability of other nutrients, e.g. phosphorus, iron, and molybdenum. The latter two make up necessary constituents of the nitrogenase.

I found that the relationship between concentration of DIN and the start of N_2 fixation was complicated by intra-lake turnover of particle-bound nitrogen (IV). It was hypothesized that even when DIN was low after the spring phytoplankton maximum, turnover of the seston-nitrogen pool could supply so much nitrogen as to inhibit dinitrogen fixation. This continued until most labile organic N was exhausted. Low $DIN:PO_4-P$ and seston $N:P$ ratios also suggested nitrogen deficiency before N_2 fixation started. The fact that seston P and PO_4-P remained low until after the N_2 -fixation maximum in Lake Trummen in 1976 suggested that P did not regulate the initial growth of N_2 fixers (IV). In Lake Södra Bergun-

dasjön P limitation was unlikely since phosphate levels were very high during summers (III).

The decline in N_2 fixation in the middle or end of summer was probably caused by different factors in different years, e.g. increasing DIN concentrations, decreasing temperature, and phosphorus or iron limitation (IV).

Diurnal and vertical variation of N_2 fixation in the water column reflected couplings between dinitrogen fixation and light-dependent metabolic processes. Nocturnal N_2 fixation was estimated to correspond to c. 15% of total 24-h nitrogenase activity (IV).

MANAGEMENT MEASURES AND N_2 FIXATION

1. Sewage-water diversion

Diversion of sewage water from Lake Södra Bergundasjön resulted in decreased nitrogen availability to phytoplankton, and non- N_2 -fixing Microcystis species were replaced by N_2 -fixing Anabaena spiroides var. crassa (III). The superabundant supply of phosphorus from the sediment was possible because of retention of this nutrient during the recipient period (Bengtsson 1978). Nitrogen is not retained by similar mechanisms.

Phytoplankton biomass and productivity did not decrease after diversion. Both Microcystis and Anabaena possess gas vacuoles, and create nuisance blooms. Consequently, the recreational value of the lake was not increased in the short term. Instead, what happened demonstrated how biological compensation mechanisms may work (cf. Schindler 1977). Thus, the sewage-diversion effects were counter-acted by the conserving effect of N_2 -fixing algae. Decreased nitrogen fixation and phytoplankton biomass is only possible if phosphorus or any other element is made growth limiting to the blue-green algae in the future. By natural release and dilution of sediment phosphorus this might take c. 20 years (Bengtsson 1978).

Schindler (1977) warned that control of external nitrogen load to lakes might be favorable to N_2 -fixing blue-greens, which were thought to further deteriorate water quality. It is assumed that the author referred to eutrophic lakes characterized by green algae, and not to Microcystis lakes. According to my knowledge, sewage diversion did not in any case result in degenerated water quality or decreased recreational values.

2. Lake restoration

The dredging of the nutrient-rich upper sediment layer in Lake Trummen resulted in decreased lake-water concentrations of most nutrients. The c. 10- and 20-fold reductions in total phosphorus and phosphate, respectively, were considered most important (IV). Phytoplankton biomass and productivity decreased, and the assemblage composition changed from large-sized blue-green algae to small-sized ones, considered typical for less eutrophic conditions (Cronberg 1982).

No nitrogen-fixation studies were performed during the pre-restoration period, but conditions similar to those in Lake Södra Bergundasjön after sewage diversion, and presence of high biomasses of Aphanizomenon flos-aquae and two Anabaena species actually carrying heterocysts suggest that N_2 fixation could have been considerable.

During the post-restoration period, N_2 fixation slowly decreased between 1973 and 1976. This was considered a result of increasing phosphorus deficiency, although occasional iron depletion could have hampered late-summer nitrogenase activity. In 1978 an extremely low biomass of heterocystous blue-green algae occurred, and N_2 fixation was almost non-existent. This was probably due to intense competition for phosphorus by small-sized Aphanothece clathrata, which formed a high biomass early in the summer. It was suggested that the predominance of this non- N_2 -fixing species was induced by a large-scale

fish-elimination experiment (performed by Dr. G. Andersson), resulting in reduced zooplankton grazing pressure on small particles (IV).

In conclusion, the restoration brought positive effects in chemical and biological parameters. During the study period successive changes took place, and the ecosystem was still not stabilized 7-8 years after the dredging. Further improvement was achievable by control of the fish assemblage.

NITROGEN BUDGETS

In Lake Södra Bergundasjön the sewage diversion resulted in a reduction of the total N input of c. 90% in 1974 (III, Bengtsson 1978). During 1975 and 1976 N_2 fixation contributed 2.2 and 4.1 g N m⁻² year⁻¹ (III, corrected for unsaturating C₂H₂ additions). During June-Sept this corresponded to c. 60 and 85% of the total nitrogen input, respectively, and over the whole years to c. 17 and 38%.

In Lake Trummen N_2 fixation supplied 3.4, 1.7, 1.9 and 0.12 g N m⁻² during 1973, 1974, 1976 and 1978, respectively (IV). The pre-restoration nitrogen fixation was roughly estimated to be in the range of 1.8-60 g N m⁻² year⁻¹. Since the hydrological budget was not balanced, the proportion of N_2 fixed to the total nitrogen input was only preliminary calculated to 24, 14, 15 and 1% for the whole years. Since Lakes Trummen and Södra Bergundasjön have similar hydrological regimes, it is assumed that N_2 fixation was as important for summer plankton development in Lake Trummen as in Lake Södra Bergundasjön.

In Lake Trummen N_2 fixation on most occasions supplied 80% or more of the estimated N demand of heterocystous algae (V). On three out of nine occasions N_2 fixation also corresponded to the requirements of the total phytoplankton assemblage. Probably due to higher

nitrogen availability during summer, the demands of heterocystous algae and the total phytoplankton assemblage were covered less frequently in Lake Södra Bergundasjön. The results suggest that heterocystous algae occasionally fix N_2 at high rates and store this for later growth, or that they assimilate DIN simultaneously or sequentially to N_2 fixation. Previously, simultaneous uptake of nitrate, ammonium and N_2 has only been shown for an Alaskan *Anabaena* population (Billaud 1968).

By integrating the total nitrogen demand during June-Sept in 1975 and 1976 in Lake Södra Bergundasjön (cf. Tab. 2 in paper V), and distributing this among N_2 - and non- N_2 -fixing algae in proportion to their biomasses, and including data on changes in lake-water, phytoplankton, non-phytoplankton-sediment and sediment nitrogen pools, tentative flow models were prepared (Fig. 1).

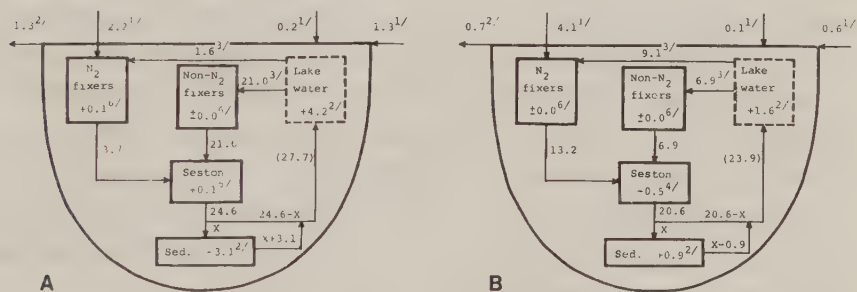


Fig. 1. Nitrogen flow models for the periods June-Sept 1975 (A) and 1976 (B) in Lake Södra Bergundasjön. Data sources: 1/ Paper III, 2/ Bengtsson (1978), 3/ Paper V, 4/ Leonardson (unpubl.), 5/ Bengtsson (unpubl.), 6/ Estimated from phytoplankton biomass changes. () denotes residual value.

The flow models emphasize the dependence of N_2 -fixing algae on dissolved inorganic nitrogen. Of their total vegetation-season N demand in 1975 and 1976 c. 40 and 70%, respectively, might have been supplied by DIN. While in 1975 the minor proportion (c. 15%) of phyto-

plankton nitrogen turnover was via heterocystous algae, this was increased to c. 65% in 1976. This indicates increasing nitrogen depletion after the sewage diversion, and successively greater importance of N_2 -fixing algae in Lake Södra Bergundasjön.

Finally, it should be stated that more frequent sampling during the N_2 -fixation periods would have been desirable. This would have increased the resolution of N_2 -fixation data, and strengthened the conclusions arrived at. However, several factors made this difficult to attain: inadequate financial support, methodological problems not resolved until 1976-77, distantly situated study lakes, and sampling programs in other lakes competing for scarce time.

FUTURE RESEARCH

In order to understand better the waxes and wanes of heterocystous blue-green algae, and improve the basis for future management measures, the controversy concerning the importance of dissolved inorganic nitrogen to heterocystous algae needs to be resolved. I suggest that the simultaneous uptake of N_2 , NH_4 and NO_3 should be studied in natural phytoplankton assemblages containing N_2 -fixing species by means of ^{13}N methodology combined with microautoradiography (cf. Wolk et al. 1974).

Until recently N_2 -fixation research has been directed towards the understanding of effects of nutrients on N_2 fixation. Biotic interactions may, however, be as important as nutrient concentrations for the regulation of N_2 -fixing algae (IV). I believe that a change in research orientation towards organism-interaction effects on heterocystous algae and N_2 fixation would be very profitable, e.g. effects of zooplankton grazing (cf. Peterson Holm et al. 1983).

TACK

Först och främst vill jag rikta ett varmt tack till mina båda handledare professor Sven Björk och docent Anders Södergren för allt stöd och all konstruktiv kritik de givit mig under avhandlingens olika faser.

Jag vill också tacka mina kolleger i Trummens och Södra Bergundasjöns forskargrupper, Gunnar Andersson, Lars Bengtsson, Gertrud Cronberg, Siegfried Fleischer, Curt Gelin och Wilhelm Ripl, för gott samarbete, värdefulla diskussioner och stor beredvillighet att alltid ställa opublicerat material till mitt förfogande.

Mina nära vänner Michael F. Coveney och Gunnar Gahnström har tålmodigt lyssnat till mina utläggningar och stimulerat mig i nästan dagliga diskussioner.

Karin Nilsson lade ner värdefullt arbete vid genomförandet av metodikstudier och löpande analyser av sestons fosfor- och kväveinnehåll.

Tack också till Amelie Fritzon för utförda planktonanalyser, till min assistent Jörgen Jonasson för gott samarbete i laboratoriet och i fält, och till Gun Jönsson för utskrifter av manuskript. Lennart Okla var snar till hjälp vid ordbehandlings- och datorproblem, och Ian Winfield språkgranskade mina sista artiklar i avhandlingen. Ulla Persson har under det sista året skött mina fältprovtagningar och avlastat mig från många arbetsuppgifter.

Sist men inte minst väsentligt: det stora stöd min fru Eva-Karin och mina pojkar Jakob och Olof alltid givit mig har varit en förutsättning för avhandlingsarbetets genomförande.

REFERENCES

- Bengtsson, L. 1978. Effects of sewage diversion in Lake Södra Bergundasjön. I. Nitrogen and phosphorus budgets. - Vatten 34:2-9.
- Billaud, V.A. 1968. Nitrogen fixation and the utilization of other inorganic nitrogen sources in a subarctic

- lake. - J. Fish. Res. Board Can. 25:2101-2110.
- Claesson, A. and S.-O. Ryding. 1977. Nitrogen - a growth limiting nutrient in eutrophic lakes. - Prog. Wat. Tech. 8:291-299.
- Cronberg, G. 1982. Phytoplankton changes in Lake Trummen induced by restoration. Long-term whole-lake studies and food-web experiments. - Folia Limnologica Scandinavica 18. 119 pp.
- Dilworth, M.J. 1966. Acetylene reduction by nitrogen-fixing preparations from Clostridium pasteurianum. - Biochim. biophys. Acta 127:285-294.
- Flett, R.J., R.D. Hamilton and N.E.R. Campbell. 1975. Nitrogen fixation in aquatic environments - a critical study of the acetylene reduction assay. - Verh. Internat. Verein. Limnol. 19:2664-2668.
- Flett, R.J., R.D. Hamilton and N.E.R. Campbell. 1976. Aquatic acetylene-reduction techniques: solutions to several problems. - Can. J. Microbiol. 22:43-51.
- Fogg, G.E., W.D.P. Stewart, P. Fay and A.E. Walsby. 1973. The blue-green algae. - Academic Press, London and New York. 459 pp.
- Peterson Holm, N., G.G. Ganf and J. Shapiro. 1983. Feeding and assimilation rates of Daphnia pulex fed Aphanizomenon flos-aquae. - Limnol. Oceanogr. 28:677-687.
- Postgate, J. 1978. Nitrogen fixation. - Studies in Biology 92. Edward Arnold Ltd, London. 67 pp.
- Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. - Science 195:260-262.
- Schöllhorn, R. and R.H. Burris. 1966. Study of intermediates in nitrogen fixation. - Fedn. Proc. 25:710.
- Stewart, W.D.P., G.P. Fitzgerald and R.H. Burris. 1967. In situ studies on N_2 fixation using the acetylene reduction technique. - Proc. natn. Acad. Sci. U.S.A. 58:2071-2078.
- Stewart, W.D.P., T. Mague, G.P. Fitzgerald and R.H. Burris. 1971. Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. - New Phytol. 70:497-509.

Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water

LARS LEONARDSON

Institute of Limnology, University of Lund, Box 3060, S- 220 03 Lund, Sweden

Accepted February 11, 1980

LEONARDSON, L. 1980. Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water. *Can. J. Microbiol.* **26**: 599–605.

Lugol's solution is a practical and efficient fixative for the acetylene-reduction assay of nitrogenase activity in aquatic organisms. Correction must be made, however, for the solubility of ethylene in the liquid phase and reactions between Lugol's solution and ethylene. With a vapor phase – liquid phase volume ratio of 1.9:1, the mean solubility of ethylene in mixtures of lake water and Lugol's solution was 7.2%. No correlation was found between ethylene solubility and the concentration of Lugol's solution. Storage of fixed samples for more than 1 day before gas chromatographic analysis resulted in increased loss of ethylene from the vapor phase; the loss amounted to ca. 18% after 3 days. Higher losses were noted at higher concentrations of Lugol's solution. Most probably these effects were caused by iodine addition to ethylene, as indicated by the consumption of ethylene by iodine – potassium iodide solutions. The reaction was catalyzed by the rubber septa of the incubation vessels when the septa were in contact with the liquid phase. Loss of ethylene decreased with increased concentration of phytoplankton because the organisms absorbed iodine. By using a standardized technique and determining ethylene solubility and reaction patterns between ethylene and the mixture of water and Lugol's solution, it is possible to correct for the loss of ethylene.

LEONARDSON, L. 1980. Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water. *Can. J. Microbiol.* **26**: 599–605.

Dans les essais de réduction d'acétylène pour déterminer l'activité nitrogénase chez les organismes aquatiques, le Lugol s'avère une solution fixatrice efficace et pratique. Une correction doit cependant être faite pour tenir compte de la solubilité de l'éthylène de la phase liquide et des réactions entre la solution de Lugol et l'éthylène. Dans une phase vapeur – phase liquide, de rapport volumétrique 1.9:1, la solubilité moyenne de l'éthylène dans des mélanges d'eau de lac et de solution de Lugol est de 7.2%. Aucune corrélation n'a été trouvée entre la solubilité de l'éthylène et la concentration de la solution de Lugol. Le remisage des échantillons fixés pour une période de plus de 1 journée avant de procéder à l'analyse chromatographique résulte en une perte plus grande l'éthylène de la phase vapeur; la perte s'élève à environ 18% après 3 jours. En outre, plus les concentrations de la solution de Lugol sont élevées, plus les pertes sont grandes. Il est fort probable que ces effets soient causés par l'addition d'iode à l'éthylène, comme cela s'est vérifié lors de la consommation d'éthylène par des solutions d'iode – iodure de potassium. La réaction fut catalysée par les cloisons en caoutchouc des fioles d'incubation, lorsque ces cloisons étaient en contact avec la phase liquide. La perte d'éthylène diminue avec une concentration accrue de phytoplancton, parce que ces organismes absorbent l'iode. Il est possible d'apporter des corrections pour la perte d'éthylène, en utilisant une technique normalisée et en déterminant la solubilité de l'éthylène, ainsi que les modes de réaction entre l'éthylène et les mélanges d'eau et de solution de Lugol.

[Traduit par le journal]

Introduction

The acetylene-reduction assay for indirect measurement of aquatic N_2 fixation is a sensitive, practical, and inexpensive method. However, as there is no accepted standardized procedure, details of the assay are quite variable among different workers. Flett et al. (1976) discussed several factors affecting the measurements negatively and suggested modifications. The effects of different fixatives were not treated, however.

The most common fixatives are 50% trichloroacetic acid (TCA), 5 N H_2SO_4 , and satu-

rated $(NH_4)_2SO_4$. The reagent is added to the sample in volumes amounting to 20–100% of the volume of the liquid phase. As the production of ethylene often is low, it is desirable to have a large liquid phase – vapor phase volume ratio in the incubation vessel to obtain higher concentrations of ethylene for gas chromatographic analysis (Flett et al. 1976). However, the addition of a large volume of fixative to the sealed vessel may be hazardous, since this increases gas pressure within the vessel and the risk of leakage during storage.

Lugol's solution, which is commonly used for

0008-4166/80/050599-07\$01.00/0

©1980 National Research Council of Canada/Conseil national de recherches du Canada

TABLE 1. Chemical and biological characteristics of the experimental lake waters

Characteristic	Lake Bysjön (August 1975)	Lake Bysjön (June 1978)	Lake Väcksjön (August 1975)
pH	9.7	9.3	9.3
Alkalinity, mequiv./L	1.99	2.76	0.52
Conductivity, $\mu\text{S}_{20}/\text{cm}$	260	345	220
Total P, mg/L	0.40	0.62	0.20
Total N, mg/L	1.76	1.11	3.14
Phytoplankton biomass, mg fresh weight/L	25	10	15
Predominant phyto- plankton species	<i>Aphanizomenon flos-aquae</i>	<i>Aphanizomenon flos-aquae</i>	<i>Anabaena spiroides</i>

preserving phytoplankton samples, was used by Lundgren (1978) and Leonardson and Bengtsson (1978) to stop acetylene reduction in field studies of periphytic and pelagic nitrogen fixation, respectively. Lännergren et al. (1974) found that fixation with Lugol's solution gave the same results as did the use of $(\text{NH}_4)_2\text{SO}_4$. Leonardson (unpublished results) found no ethylene production in samples fixed with Lugol's solution, despite production of up to 8×10^{-9} mol $\text{C}_2\text{H}_4/\text{mL}$ lake water in unfixed samples. This verifies that acetylene reduction is efficiently inhibited by the addition of Lugol's solution. As Lugol's solution also allows the advantages of the addition of a small volume of fixative to the sample and the possibility of making algal counts directly from the contents of the incubation vessel, it is a desirable fixative. However, losses of ethylene due to solubility in the liquid phase and chemical reactions are to be expected. The purpose of this investigation was to determine the extent of these losses and the effects of phytoplankton concentrations and storage conditions.

Materials and methods

Water from Lake Bysjön in Scania, Sweden (June, 1978) was used in all experiments but one: the experiment testing the effects of phytoplankton concentration on the loss of ethylene was performed in August, 1975, in Lake Bysjön and Lake Väcksjön, Småland, Sweden. Lake water data from these occasions are presented in Table 1. Additional information on the lakes is found in Coveney et al. (1977) and Bengtsson and Gelin (1975).

Lake water (5 mL) was pipetted into 15-mL test tubes (soda-glass) which were closed with butyl-rubber septa and screw caps. After injection of 0.15 mL Lugol's solution (10 g iodine, 20 g potassium iodide, 200 g distilled water, 20 g glacial acetic acid; Willén 1962) through the septa into the closed test tubes with a syringe, the samples were agitated. When lake water without fixative made up the liquid phase (5.15 mL), the water was autoclaved, cooled to room temperature, and bubbled with air for gas equilibration before being added to the test tubes. Finally, 1.0 mL of an ethylene-acetylene mixture was injected with a syringe. To estimate the percentage loss of ethylene to the liquid phase, test tubes with vapor phase only (air, ethylene, acetylene) were included in each experiment. The test tubes

were stored upright at room temperature (ca. 20°C) in the dark and were agitated for 60 s before removal of gas for analysis, if not stated otherwise.

Ethylene in 1.0 mL of the vapor phase of the tubes was determined by gas chromatography with a flame ionization detector (Varian series 3700) using a 2.7-m stainless steel column (1/16 in. (1 in. = 25.4 mm)) packed with Porapak T. The temperature of the column oven was 90°C. N_2 was used as carrier gas at a flow rate of 30 mL/min. Results are expressed in millimetres peak height per millilitre gas sample. Peak height and peak area were shown to be proportional in the range 10–200 mm or 3×10^{-11} – 5×10^{-10} mol C_2H_4 . The standard deviation of 10 replicate analyses of calibration gas (2.0×10^{-10} mol $\text{C}_2\text{H}_4/\text{mL}$) was 0.8 mm at a mean peak height of 104 mm. The minimum detectable quantity was 4×10^{-12} mol C_2H_4 .

Ethylene concentration in the tubes was calculated using the total vapor phase volumes (test tube and syringe). The small excess of pressure caused by the addition of 0.15 mL of Lugol's solution to the closed tubes was considered. The percentage loss of ethylene in each experimental series was determined by comparing the vapor phase ethylene concentrations in tubes with and without liquid phase. In the statistical treatment results were assumed to be normally distributed and Student's *t*-test was used to compare sample means. Confidence limits (95%) of ethylene loss (in percentage units) were estimated with Gauss' error propagation law.

Phytoplankton counts were made by the Utermöhl technique. Biomass (milligrams fresh weight per litre) was calculated from cell volume determinations assuming a specific weight of 1.0 (cf. Willén 1976). A range of 150–500 organisms was counted in every sample. The χ^2 test was used to check for random organism distribution on the counting chamber bottom (Lund et al. 1958). The replicate test tubes for each phytoplankton concentration tested were pooled for phytoplankton biomass determination. Only N_2 -fixing blue-green algae were counted in concentrated samples as other algae made up only 0.2 and 8% of the total biomass in unconcentrated samples from Lake Bysjön and Lake Väcksjön, respectively. To facilitate counting, algae from Lake Väcksjön were treated with ultrasound.

Results

Test of nonbiological production of ethylene

Three test tubes containing a mixture of tap water and Lugol's solution with air in the vapor phase were closed with rubber septa. The test tubes were stored upside down, so that the liquid phase was in contact with the septa, and agitated frequently. After 16 h, 15 days, and 19 days, 0.5 mL gas

from each of the tubes was analysed. No ethylene or acetylene was detected.

Immediate loss of ethylene from the vapor phase to the liquid phase

Samples containing ethylene-acetylene-air and either a mixture of water and Lugol's solution or only water were agitated for 5, 20, 40, 80, and 300 s and the final concentration of ethylene in the vapor phase was determined (Fig. 1). Agitation time did not affect the transfer of ethylene from the vapor phase to the liquid phase ($p > 0.05$). The loss of ethylene to the mixture of water and Lugol's solution was $7.2 \pm 1.5\%$, and to water, $2.4 \pm 1.5\%$ (95% confidence limits).

Long-term loss of ethylene from the vapor phase to the liquid phase

Loss of ethylene from the vapor phase over a 14-day period was studied in test tubes containing water and Lugol's solution, water, and vapor phase only. The experiment was performed at three different ethylene concentrations.

During the first 3 days of storage, ethylene concentrations decreased rapidly in the vapor phase of the test tubes containing a mixture of water and Lugol's solution. After this period the decrease was slower and approximately linear (Fig. 2). To compare data from the three series of ethylene concentrations, the values were expressed as a percentage of the mean ethylene concentration on day 0 for each series (Fig. 3). No significant differences were found between the arithmetic means of the three series on the separate days of analysis ($p > 0.05$). However, as the variance of values from the series with the lowest ethylene concentration added was high, these measurements were excluded when estimating the mean total loss of ethylene.

Ethylene concentrations in test tubes containing

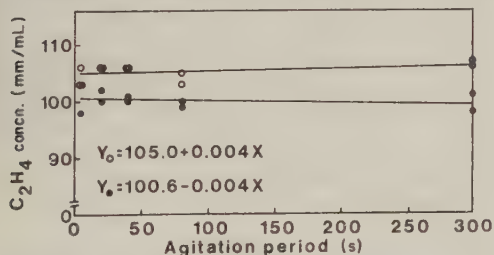


FIG. 1. Effect of different agitation periods on ethylene concentration in the vapor phase of test tubes containing water (O, $n = 10$) and water and Lugol's solution (●, $n = 10$). Regression equations for the two lines are given. The ethylene-acetylene mixture added to the samples contained 4.8×10^{-9} mol C_2H_4 /mL. Test tubes containing vapor phase only ($n = 4$) showed peak heights ranging from 73 to 75 mm/mL ($\bar{x} = 74.3$ mm/mL).

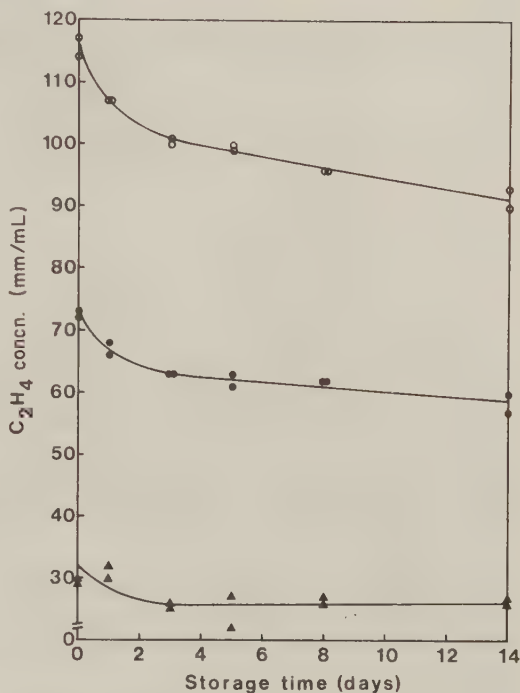


FIG. 2. Long-term decrease in ethylene concentration in the vapor phase of test tubes containing a mixture of water and Lugol's solution. The ethylene-acetylene mixture added to the samples contained 6.6×10^{-11} (▲, $n = 12$), 3.4×10^{-9} (●, $n = 12$), and 2.2×10^{-7} (○, $n = 12$) mol C_2H_4 /mL.

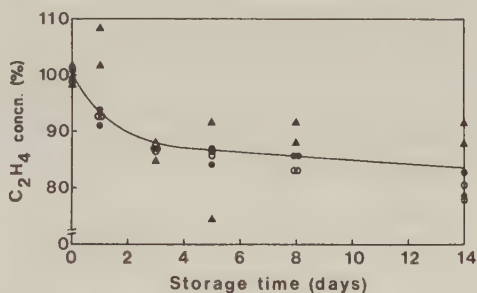


FIG. 3. Long-term decrease in ethylene concentration in the vapor phase of test tubes containing a mixture of water and Lugol's solution and different ethylene concentrations (cf. Fig. 2). Values expressed as a percentage of ethylene concentration on day 0 for each series.

water as liquid phase did not decrease significantly after day 0 (Table 2). The immediate loss of ethylene amounted to $2.5 \pm 1.8\%$ (95% confidence limits).

In test tubes containing only vapor phase, ethylene decreased significantly at the intermediate concentration added. For pooled data from the

TABLE 2. Regression equations describing changes during long-term storage in ethylene concentration in the vapor phase of test tubes containing water as liquid phase and of test tubes without liquid phase^a

Contents of test tubes	Concn. of C ₂ H ₄ added, mol/mL	Regression equation	95% confidence limit	n
Water phase	6.6×10^{-11}	$y = 31.9 + 0.01x$	0.15	12
Water phase	3.4×10^{-9}	$y = 75.6 - 0.13x$	0.20	12
Water phase	2.2×10^{-7}	$y = 121.7 - 0.42x$	0.60	11
Pooled data ^b	—	$y = 99.9 - 0.15x$	0.26	35
No liquid phase	6.6×10^{-11}	$y = 22.5 - 0.00x$	0.16	11
No liquid phase	3.4×10^{-9}	$y = 54.2 - 0.26x^c$	0.15	12
No liquid phase	2.2×10^{-7}	$y = 85.4 - 0.18x$	0.36	11
Pooled data ^b	—	$y = 99.5 - 0.22x$	0.28	34

^aConfidence limits (95%) of the regression coefficients are given. Analyses of ethylene were made on days 0, 1, 3, 5, 8, and 14.

^bValues expressed as percentage of ethylene concentration on day 0 for each series.

^cRegression coefficient significantly different from zero ($p < 0.05$).

three series of ethylene concentrations, ethylene loss was calculated to ca. 0.2% per day, and the regression coefficient was not significantly different from zero (Table 2).

The mean loss of ethylene from the vapor phase to the mixture of water and Lugol's solution amounted to 8.5% immediately upon starting the experiment (day 0) and 22% after 14 days of storage (Fig. 4).

Effects of different mixtures of water and Lugol's solution on the loss of ethylene

Increasing concentrations of Lugol's solution in the liquid phase did not significantly affect the ethylene content of the vapor phase within the first hours of the storage period (day 0, Table 3). The mean loss of ethylene for the four series was $8.6 \pm 1.8\%$ (95% confidence limits). After 8 days of storage there was a significantly greater loss of ethylene from the vapor phase with increasing concentration of Lugol's solution ($p < 0.001$). The loss of ethylene ranged from 19.4 to 38.7%.

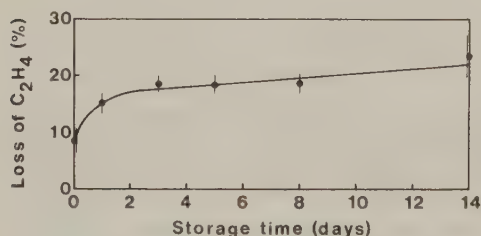


FIG. 4. Percentage loss of ethylene from the vapor phase of test tubes containing a mixture of water and Lugol's solution during long-term storage. Arithmetic mean and 95% confidence limits are shown. Curve fitted by eye. The values were taken from two experimental series with 3.4×10^{-9} ($n = 12$) and 2.2×10^{-7} ($n = 12$) mol C₂H₄/mL added to the test tubes.

TABLE 3. Loss of ethylene from the vapor phase to varying mixtures of water and Lugol's solution within the first hours after the start of the experiment (day 0) and after 8 days of storage^a

Volume % Lugol's solution	Loss of ethylene, %	
	Day 0	Day 8
2.9	8.0 ± 1.8	19.4 ± 1.8
9.7	8.7 ± 2.1	27.2 ± 1.7
19.4	9.1 ± 2.2	32.8 ± 1.5
38.8	8.7 ± 2.0	38.7 ± 2.6

NOTE: The ethylene-acetylene mixture added to the samples contained 6.5×10^{-9} mol C₂H₄/mL. Test tubes containing only vapor phase ($n = 12$) were included in the experiment.

^aArithmetic mean and 95% confidence limits are given; $n = 6$ in each series.

Chemical reactions between ethylene and Lugol's solution

To study whether the long-term loss of ethylene from the vapor phase was caused by reactions between ethylene and iodine plus potassium iodide or ethylene and acetic acid, these reagents were mixed separately with water in two series.

The loss of ethylene from the vapor phase to the mixture of water and iodine plus potassium iodide was low on the first day of storage but increased significantly ($p < 0.001$) during 1 week (Table 4). The loss of ethylene to the mixture of water and acetic acid was low and did not change during storage ($p > 0.05$).

Effects of rubber septa and storage temperature on the ethylene concentration in the vapor phase

To study effects of alternative storage conditions on samples, test tubes were incubated at 5 and 20°C both upright and upside down, so that the liquid phase in the latter case was in contact with the rubber septum (Table 5).

TABLE 4. Loss of ethylene from the vapor phase to mixtures of water and iodine plus potassium iodide and acetic acid, respectively^a

Liquid phase	Loss of ethylene, %	
	Day 0	Day 8
Water-I ₂ -KI	4.8 ± 1.8	16.7 ± 1.9
Water - acetic acid	7.3 ± 1.7	6.8 ± 2.3

NOTE: Additions (0.15 mL) of iodine - potassium iodide in distilled water and aqueous acetic acid, both solutions at the same concentrations as in 2.9% Lugol's solution, were made to the test tubes. The ethylene-acetylene mixture added contained 6.4×10^{-9} mol C₂H₄/mL. Test tubes containing only vapor phase ($n = 12$) were included in the experiment.

^aArithmetic mean and 95% confidence limits are given; $n = 6$ in each series.

At 20°C the loss of ethylene from the vapor phase to the mixture of water and Lugol's solution was significantly higher in test tubes kept upside down than in test tubes kept upright ($p < 0.001$). In test tubes kept upside down there was a greater loss at 5°C than at 20°C ($p < 0.05$). Loss of ethylene from the vapor phase of test tubes containing only water and stored upside down at 5°C did not differ significantly from that of earlier experiments ($p > 0.05$).

Effects of phytoplankton concentration on the loss of ethylene from the vapor phase

Phytoplankton from Lake Bysjön and Lake Väckjösjön, south Sweden, were concentrated with plankton nets (mesh size 10 µm) and pipetted into test tubes. Lugol's solution (0.15 mL) and 1.0 mL ethylene-acetylene mixture were added to both unconcentrated and concentrated samples, which were then stored for 13 and 8 days, respectively.

The ethylene concentration of the vapor phase increased with increasing phytoplankton concentration in both series. The data were described by the regressions $y = 36.5 + 0.002x$ ($r^2 = 0.79$) for Lake Bysjön and $y = 112.5 + 0.011x$ ($r^2 = 0.77$) for Lake Väckjösjön, where y is ethylene concentration in the vapor phase expressed as millimetres peak

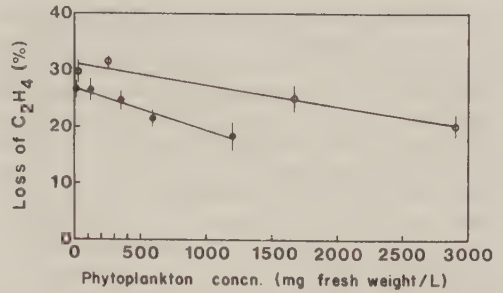


FIG. 5. Loss of ethylene from the vapor phase to a mixture of water and Lugol's solution at different phytoplankton concentrations; Lake Bysjön (○, $n = 20$) and Lake Väckjösjön (●, $n = 30$), south Sweden. Arithmetic mean and 95% confidence limits are shown. Organisms were concentrated with plankton nets, mesh size 10 µm. The ethylene-acetylene mixture added to the samples contained 5.9×10^{-10} (○) and 4.6×10^{-9} (●) mol C₂H₄/mL. Test tubes containing only vapor phase were included in the experiment ($n = 6$ in both series). Test tubes were stored upside down at 5°C for 13 (○) and 8 (●) days.

height per millilitre and x is phytoplankton biomass expressed as milligrams fresh weight per litre. The slopes of the two regressions were significantly different from zero ($p < 0.001$) and from each other ($p < 0.001$). Consequently, the loss of ethylene from the vapor phase to the mixture of water and Lugol's solution decreased significantly, but at different rates, for the two series with increasing phytoplankton concentration (Fig. 5).

Discussion

The solubility of ethylene in the liquid phase of samples cannot be neglected if a realistic assessment of aquatic N₂ fixation is desired (Sugahara and Kawai 1971; Flett et al. 1976). As ethylene is unsaturated and highly reactive with many inorganic and organic substances, care has to be taken as to the reagents used to inhibit the nitrogenase enzyme. The reduction of acetylene to ethylene will be underestimated if derivatives of ethylene are formed after the addition of fixative. On the other hand, Thake and Rawle (1972) found that ethylene

TABLE 5. Effects of rubber septa and temperature on the loss of ethylene from the vapor phase to a mixture of water and Lugol's solution after 8 days of storage^a

Liquid phase	Position of test tubes	Temperature, °C	Loss of ethylene, %
Water-Lugol	Upright	+20	24.9 ± 0.9
Water-Lugol	Upside down	+20	28.4 ± 1.4
Water-Lugol	Upside down	+5	30.5 ± 1.1
Water	Upside down	+5	2.7 ± 2.0

NOTE: The ethylene-acetylene mixture added to the samples contained 2.0×10^{-9} mol C₂H₄/mL. Test tubes containing only vapor phase were incubated at 5°C and 20°C ($n = 12$).

^aArithmetic mean and 95% confidence limits are given; $n = 6$ in each series.

was produced nonbiologically as a result of contact between trichloroacetic acid and rubber liners. They found similar effects with HCl and H₂SO₄.

The present study showed that the reaction observed by Thake and Rawle (1972) did not occur at low concentrations of Lugol's solution in contact with butyl-rubber septa.

The immediate loss of ethylene to the mixture of water and Lugol's solution ($7.2 \pm 1.5\%$) corresponds to the solubility of ethylene. This was confirmed by the fact that the loss of ethylene on day 0 was independent of the concentration of Lugol's solution (Table 3). During longer storage periods the loss of ethylene increased to about 20%. As similar loss patterns were achieved at three different concentrations of ethylene, both the immediate and long-term losses were apparently proportional to the partial pressure of ethylene in the vapor phase. The sum effect of diffusion of ethylene from the test tubes and adsorption of ethylene to the glass walls was linear and of a magnitude of 0.0–0.4% per day as estimated from test tubes containing water or only vapor phase.

The results indicate that both solubility and chemical reactions contribute to the decrease of the ethylene content of the vapor phase during long-term storage. It is proposed that iodine addition to ethylene was responsible for the chemical loss (Table 4). After 8 days of storage an increased loss of ethylene with increasing concentration of Lugol's solution was observed (Table 3), indicating that the rate of the long-term reaction of ethylene with iodine is sensitive to the concentration of Lugol's solution.

There was no significant difference in the loss of ethylene to acetic acid from the 1st to the 8th day of storage, which indicates either ethylene solubility only or a quick but negligible reaction to ethyl acetate. In addition there was no relationship between concentration of Lugol's solution in the liquid phase and loss of ethylene from the vapor phase on day 0 (Table 3). These results indicate that there was no reaction between ethylene and acetic acid in the experiments.

Influences of rubber septa, storage temperature, and organism concentration in the sample must be considered in any acetylene-reduction assay using Lugol's solution as a preservative. When incubation vessels were kept upside down so that the mixture of water and Lugol's solution was in contact with the rubber septa during storage, the loss of ethylene was significantly increased, probably because of the rubber septa acting as catalysts to the halogen addition discussed above. The loss of

ethylene was higher at 5°C than at 20°C. Generally, the solubility of gases in liquids increases at lower temperatures. Hence, at 5°C more ethylene was dissolved than at 20°C which resulted in a faster reaction between iodine and ethylene (cf. Fig. 2). In the control series with only water as the liquid phase, the loss of ethylene after storage at 5°C was not significantly different from the loss obtained at 20°C in earlier experiments because no chemical reaction occurred. As the ethylene concentration was always determined after the cooled samples were warmed to room temperature, differences in ethylene solubility at varying temperatures did not affect the analyses.

When the concentration of phytoplankton increased in the samples, the loss of ethylene to the mixture of water and Lugol's solution decreased. Phytoplankton organisms absorb iodine and reduce the amount available for reaction with ethylene. There were different patterns of ethylene loss in samples from Lake Bysjön and Lake Väckjösjön. Either the absorption capacity for iodine was different in the different phytoplankton communities or other factors, e.g., lake water composition, influenced the results (Table 1).

The loss of ethylene to mixtures of water and Lugol's solution is overcome by using a standardized technique and storing samples under defined conditions until analysis. The true ethylene production in the incubation vessel can then be calculated after determination of solubility and long-term reaction patterns for the actual experimental design.

Acknowledgements

I thank Professor S. Björk, Drs. W. Ripl and A. Södergren, and Mr. M. F. Coveney for valuable criticism of the manuscript. Miss A. Fritzon did the phytoplankton analyses and Mr. T. Persson gave statistical advice.

- BENGTSOON, L., and C. GELIN. 1975. Artificial aeration and suction dredging methods for controlling water quality. Proceedings of a symposium on the effects of storage on water quality, Reading University, March 1975. The Water Research Centre, Medmenham, England. pp. 313–342.
- COVENEY, M. F., G. CRONBERG, M. ENELL, K. LARSSON, and L. OLOFSSON. 1977. Phytoplankton, zooplankton and bacteria—standing crop and production relationships in a eutrophic lake. *Oikos*, 29: 5–21.
- FLETT, R. J., R. D. HAMILTON, and N. E. R. CAMPBELL. 1976. Aquatic acetylene-reduction techniques: solutions to several problems. *Can. J. Microbiol.* 22: 43–51.
- LÄNNERGREN, C., A. LUNDGREN, and U. GRANHALL. 1974. Acetylene reduction and primary production in Lake Erken. *Oikos*, 25: 365–369.
- LEONARDSON, L., and L. BENGTSOON. 1978. Effects of sewage

- diversion in Lake Södra Bergundasjön. II. Phytoplankton changes and the role of nitrogen fixation. *Verh. Int. Ver. Limnol.* **20**: 2701–2707.
- LUND, H. W. G., C. KIPLING, and E. D. LE CREN. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, **11**: 143–170.
- LUNDGREN, A. 1978. Nitrogen fixation induced by phosphorus fertilization of a subarctic lake. *Ecol. Bull. (Stockholm)*, **26**: 52–59.
- SUGAHARA, I., and A. KAWAI. 1971. Microbiological studies on nitrogen fixation in aquatic environments. V. Modification of acetylene method for the measurement of in situ rate of nitrogen fixation. *Bull. Jpn. Soc. Sci. Fish.* **37**: 1088–1092.
- THAKE, B., and P. R. RAWLE. 1972. Non-biological production of ethylene in the acetylene reduction assay for nitrogenase. *Arch. Mikrobiol.* **85**: 39–43.
- WILLÉN, E. 1976. A simplified method of phytoplankton counting. *Br. Phycol. J.* **11**: 265–278.
- WILLÉN, T. 1962. Studies on the phytoplankton of some lakes connected with or recently isolated from the Baltic. *Oikos*, **13**: 169–199.

Effects of concentrating phytoplankton on the acetylene-reduction assay for nitrogenase activity

LARS LEONARDSON Institute of Limnology, University of Lund, Sweden

SUMMARY. 1. The consequences of concentrating freshwater phytoplankton communities prior to measurement of nitrogenase activity using the acetylene-reduction assay were investigated.

2. Retention of heterocystous blue-green algae by 10 and 45 μm plankton nets was usually much less than 100% and the per cent retention differed among blue-green algal species and varied for the same species both on different sampling dates and in different lakes. Retention of heterocyst numbers and heterocyst volume during concentration differed from that of total cell volume of heterocystous blue-green algae.

3. Effects of concentration on specific nitrogenase activity varied among the lakes studied. In Lake Bysjön activity decreased on all sampling occasions, in Lake Trummen there was no apparent effect, and in Lake Väcksjön both results were found.

4. Effects on nitrogenase activity of mechanical disturbance during concentration and of increased pH, reduced carbon availability, light inhibition and subsaturation, photorespiration and oxygen supersaturation in concentrated samples are discussed. Interactions between these factors are suggested to explain the varying responses in the three lakes.

5. It is concluded that phytoplankton should not be concentrated in the acetylene-reduction assay for nitrogenase activity, since both volumetric and cell concentration factors are inadequate and effects of concentration on specific nitrogenase activity are not predictable.

Introduction

The acetylene-reduction technique for measurement of biological nitrogen fixation is an inexpensive and convenient method, often felt to give easily interpretable results. To obtain detectable amounts of ethylene from acetylene reduction in *in-situ* studies, it was suggested that phytoplankton should be concentrated before assay (Stewart, Fitzgerald & Burris, 1967; Burris, 1972). As a result, a great

variety of filter and centrifugation techniques were used (e.g. Rusness & Burris, 1970; Brooks *et al.*, 1971; Granhall & Lundgren, 1971; Findley, Findley & Stein, 1973; Vanderhoef, 1976; Torrey & Lee, 1976; Lean *et al.*, 1978; Ashton, 1979; Ahmad, 1981). Different ways of calculating natural nitrogenase activity were also employed. Some workers considered the ratio between initial and final sample volume, while others compared counts of nitrogen-fixing organisms in concentrated and unconcentrated samples to reach a conversion factor.

Decisions whether to concentrate phytoplankton or not were based on very few

Correspondence: Dr Lars Leonardson, Institute of Limnology, University of Lund, Box 3060, S-220 03 Lund, Sweden

experimental data. Stewart *et al.* (1967, 1971) reported that acetylene reduction by phytoplankton was not negatively affected in short-term studies even at high concentration factors. Horne & Goldman (1972) and Flett, Hamilton & Campbell (1976) felt that concentration procedures should be avoided, and Brattberg (1977) reported slightly decreased specific nitrogenase activity in field experiments with concentrated samples. However, no thorough investigations have been performed to assess the consequences of concentration procedures.

In this study an evaluation of the effects of concentrating phytoplankton for the acetylene-reduction assay is presented. The efficiency of concentration was determined for various heterocystous blue-green algae, and the dilemma of whether concentration factors should be based on total cell volume, number of heterocysts or heterocyst volume was considered. Finally, effects of concentration on specific nitrogenase activity were investigated in different phytoplankton communities.

Materials and Methods

Sampling sites

Lake water and phytoplankton were sampled in the pelagic zone of four meso- to eutrophic lakes in southern Sweden: Lake Bysjön, Lake Trummen, Lake Vombsjön and Lake Våxjösjön (Enell, 1980; Gelin & Ripl, 1978; Gelin, 1975; Bengtsson & Gelin, 1975). Summer phytoplankton communities were characterized by regularly-occurring blooms of nitrogen-fixing blue-green algae of the genera *Anabaena* and *Aphanizomenon*.

Sampling procedure

Water for the acetylene-reduction assay was collected at 0.0–0.4 m depth. 1.3–5.0 l were filtered through conical plankton nets with the length of square sides of mesh openings 10 μm ('10 μm net') or 45 μm ('45 μm net'). The filtering procedure took about 5 min. Water for unconcentrated samples was taken either as a separate sample (1973–74) or from the sample described above before concentration (1975–77).

Experimental procedure

A modified version of the acetylene-reduction technique (Stewart *et al.*, 1967) was

used. 5.0 ml lake water was pipetted into 15.0 ml test tubes closed with butyl-rubber septa and screw caps. These were kept in a shaded area of the boat until incubation. If not stated otherwise, 1.0 ml acetylene (scrubbed in two traps of concentrated H_2SO_4 and a water trap; Hardy, Burns & Holsten, 1973) was injected into each test tube by slowly bubbling the gas through the organism suspension. This gas amount corresponds to a theoretical solubility of 0.07 ml C_2H_2 ml^{-1} H_2O (Flett *et al.*, 1976). The experiments were carried out in triplicate. One 'blank' tube containing organisms preserved with 0.15 ml Lugol's solution was incubated for each organism concentration. Incubations were made *in situ*, usually from 11.30 to 13.30 hours, after which acetylene reduction was stopped by injection of 0.15 ml Lugol's solution. Test tubes were stored upside-down in the dark at 5°C for up to 9 days. Loss of ethylene from the vapour phase due to solubility and chemical reaction with Lugol's solution was considered (Leonardson, 1980). Samples were equilibrated to room temperature and agitated 30–60 s before removal of gas for analysis.

Ethylene analysis

1.0 ml gas from the test tubes was analysed for ethylene production by a gas chromatograph with a flame ionization detector (Varian Aerograph, series 1520), using a 2.7 m stainless steel column (1.6 mm inner diameter) packed with Porapak T and operated at 90°C. Nitrogen was utilized as carrier gas at a flow rate of 30 ml min^{-1} . Coefficients of variation for six replicate samples of calibration gas (2.8×10^{-10} mol C_2H_4 ml^{-1}) were less than 1.8% at mean peak heights between 116 and 125 mm. The minimum detectable quantity varied between 3.9×10^{-12} and 6.5×10^{-11} mol C_2H_4 .

Phytoplankton cell counts

Aliquots of phytoplankton suspension were removed from the individual incubation vessels used for acetylene-reduction measurements and counted. 100–500 colonies and/or cells of dominating species of heterocystous blue-green algae were counted with the inverted microscope technique (Utermöhl, 1958). Counts of non-heterocystous algae were restricted to

about 100 organisms. To obtain more accurate cell numbers, phytoplankton colonies in samples from Lake Bysjön and Lake Väcksjön, which contained only one heterocystous blue-green algal species, were broken by ultrasonic treatment (cf. Cronberg, 1982). The chi-square test was used to check for random organism distribution in the counting chamber (Lund, Kipling & Le Cren, 1958).

In the determination of total cell volume ($\text{mm}^3 \text{ l}^{-1}$) of *Aphanizomenon* species, filament length ($n=100-500$) and cross-sectional area of individual cells ($n=10$) were measured. For *Anabaena* species treated with ultrasound, the number of cells either free or in short chains ($n=100-500$) and volume per cell ($n=10-20$) were estimated. Otherwise, total number of trichome spirals plus separate cells ($n=100-500$), number of cells per spiral ($n=10-20$), and volume per cell ($n=10-20$) were measured to give the total volume of *Anabaena*. Akinetes were not included in total cell volume estimates. The variance in total cell number and total cell volume was estimated from the variance of separate counts, e.g. number of spirals, number of cells per spiral and volume per cell for *Anabaena*.

Concentration factors

Two types of conversion factors were used to estimate natural lake-water nitrogenase activity from measurements in concentrated organism suspensions. The volumetric concentration factor was defined as the ratio between initial and final sample volume. Cell concentration factors were defined as ratios between cell volume or cell number of heterocystous algae in the concentrated sample and the equivalent values in unconcentrated lake water. Per cent retention of algae in plankton nets was calculated as cell concentration factor $\times 100$ / volumetric concentration factor. Specific nitrogenase activity was defined as ethylene production per cell volume or cell number per unit time.

Water chemistry

pH was measured potentiometrically with a pH meter PHM 29 (Radiometer, Copenhagen, Denmark) equipped with a combination electrode.

Total alkalinity was estimated by titration with 0.01 N HCl in a nitrogen-flushed chamber

with a mixed pH indicator. Carbonate alkalinity was calculated according to Golterman, Clymo & Ohnstad (1978), and distribution of dissolved inorganic carbon species was estimated according to Buch (1945). The equilibrium constants of Buch were corrected for water temperature (Golterman *et al.*, 1978).

Results

Retention of heterocystous blue-green algae by plankton nets

Concentration of phytoplankton communities with 10 μm plankton nets resulted in varying retention of heterocystous blue-green algal cell volume (Fig. 1). In twenty-four of thirty-nine experiments, retention was between 40% and 80%. Retention by a 45 μm plankton net varied between 3% and 14% on four sampling dates, and reached 42% once. No statistically significant relationship ($P>0.05$; linear regression analysis and Spearman's rank correlation) was found between per cent retention and the volume of filtered water, volumetric concentration factor or total cell volume of the unconcentrated phytoplankton community. Relationships were still not significant after grouping of data by lakes.

Retention of heterocystous blue-green algae varied greatly in both Lakes Trummen and Bysjön (Table 1). *Anabaena spiroides* var.

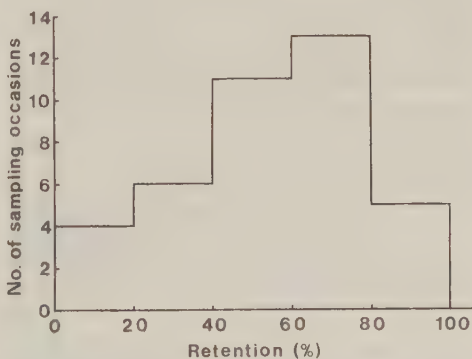


FIG. 1. Frequency distribution of percentage retention of heterocystous blue-green algal cell volume during concentration with 10 μm plankton net. Mean of microscopic counts from two to four depths on sampling occasions in Lake Bysjön ($n=11$), Lake Trummen ($n=16$), Lake Vombsjön ($n=7$) and Lake Väcksjön ($n=5$).

TABLE 1. Per cent retention for cell volume of heterocystous blue-green algal species by 10 μ m plankton net, volume of total phytoplankton community and of heterocystous algae (N₂-fix) and volumetric concentration factors

Lake/Date	Total* (mm ³ l ⁻¹)	N ₂ -fix (mm ³ l ⁻¹)	Vol. conc. factor	<i>Anabaena</i> <i>spiroides</i>	<i>Anabaena</i> <i>lemmermannii</i>	<i>Anabaena</i> <i>viguieri</i>	<i>Aphani-</i> <i>zomenon</i> <i>gracile</i>	<i>Aphani-</i> <i>zomenon</i> <i>flos-</i> <i>aquae</i>
Trummen								
16 Aug. 1973	6.9	4.4	64	35	21	—	7.7	—
4 Sept. 1973	5.3	0.92	64	35	22	—	7.8	—
7 Aug. 1974	11	5.1	100	77	70	29	17	—
19 Aug. 1974	13	9.1	100	94	29	41	22	—
10 Sept. 1974	8.9	4.2	78	88	53	—	28	—
19 Sept. 1974	6.7	3.9	100	52	—	—	10	—
Bysjön								
26 Sept. 1973	4.1	3.5	64	—	71	—	—	—
22 July 1974	10	4.7	100	—	70	—	—	59
29 July 1974	11	7.2	100	—	75	—	—	100
13 Aug. 1974	26	11	100	—	—	—	—	100
26 Aug. 1974	100	100	52	—	—	—	—	100

— Species not present in the phytoplankton community.

* Volume of non-heterocystous algae in Lake Trummen from Cronberg (1982), and in Lake Bysjön from Cronberg (unpubl.).

spiroides Kleb. had higher retention than other heterocystous species in Lake Trummen, while *Aphanizomenon gracile* Lemm. showed low retention values. Retention of the same species differed both in the same lake and among different lakes (e.g. *Anabaena lemmermannii* P. Richt., Table 1).

Heterocyst retention, based on both number and volume, was lower than that of vegetative cells ($P < 0.001$ and $P < 0.05$, respectively; variance of retention values estimated according to Gauss' error propagation law, and differences between groups of data tested with two-tailed Student's *t*-test), resulting in lower concentration factors and higher specific nit-

rogenase activity (Table 2). However, retention efficiency was not significantly higher for heterocyst volume than for heterocyst number ($P > 0.05$).

Specific nitrogenase activity in unconcentrated and concentrated samples

Specific nitrogenase activity, based on total cell volume of heterocystous blue-green algae, decreased as the concentration of algae increased to 300 mm³ l⁻¹ in surface samples from Lake Bysjön. Thereafter no significant change was found (Fig. 2 and other experiments not shown). Potential nitrogen fixers were

TABLE 2. Per cent retention of total cell volume, number of heterocysts and heterocyst volume for heterocystous blue-green algae in Lake Trummen surface samples.* Values in parentheses are quotients between specific nitrogenase activity in concentrated and unconcentrated samples based on total cell volume, number of heterocysts and heterocyst volume.

Date	Total cell volume	Per cent retention No. of heterocysts	Heterocyst volume
7 Aug. 1974	53 (0.93)	40 (1.22)	43 (1.13)
19 Aug. 1974	52 (1.17)	31 (1.91)	37 (1.66)
10 Sept. 1974	51 (1.07)	44 (1.24)	49 (1.11)
19 Sept. 1974	22 (1.03)	11 (2.06)	15 (1.50)

* At least 400 colonies and/or cells and heterocysts counted. Concentration factors as in Table 1.

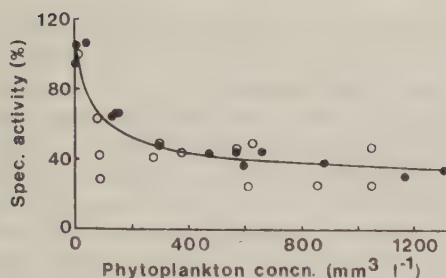


FIG. 2 Specific nitrogenase activity in per cent of unconcentrated samples as a function of heterocystous blue-green algal cell volume for two concentration series (10 μm net) in Lake Bysjön on 12 August 1975. Series I ($\circ$) was incubated at 12.10–14.10 hours and series II ($\bullet$) at 13.50–15.50 hours. 100% corresponds to 10 and 8.2 $\text{nmol C}_2\text{H}_4 \text{ mm}^{-3} 2 \text{ h}^{-1}$ for series I and II, respectively. Each point represents one ethylene and one volume ($n \geq 400$) determination from the same test tube, except for the unconcentrated sample in series I which is the mean of three ethylene measurements and one algal count from a pooled sample from the three test tubes. One obviously erroneous value in series II (467%) is omitted. *Aphanizomenon flos-aquae* made up c. 100% of the total phytoplankton volume. Curve fitted by eye.

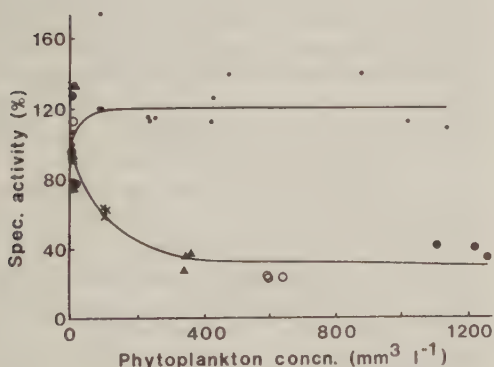


FIG. 3. Specific nitrogenase activity in per cent of unconcentrated samples as a function of heterocystous blue-green algal cell volume for two concentration series (10 μm net) in Lake Våxjösjön on 27 August 1975. Series I ($\bullet$) was subsampled from a single water sample and incubated at 12.20–14.30 hours. Series II was subsampled from four water samples so that paired unconcentrated and concentrated samples were obtained (volumetric concentration factors 0 and 10 ($\times$), 0 and 25 ($\blacktriangle$), 0 and 50 ($\circ$), 0 and 100 ($\bullet$)). These samples were incubated successively for 2 h during 13.15–16.25 hours. 100% corresponds to 56 and 79 $\text{nmol C}_2\text{H}_4 \text{ mm}^{-3} 2 \text{ h}^{-1}$ for series I and II, respectively. Each point represents one ethylene and one volume ($n \geq 400$) determination from the same test tube. The dominating alga *Anabaena spiroides* var. *spiroides* made up c. 90% of the total phytoplankton volume. Curves fitted by eye.

Aphanizomenon flos-aquae (L.) Ralfs and *Anabaena lemmermannii*.

In Lake Våxjösjön two concentration experiments were performed on the same day (Fig. 3). Series I showed slightly higher specific nitrogenase activity in concentrated samples than in unconcentrated ones. In series II there was a similar pattern of decrease in specific activity as described for Lake Bysjön. The only heterocystous alga found was *Anabaena spiroides* var. *spiroides* which made up c. 90% of the total phytoplankton volume. Other algae present were *Oscillatoria agardhii* Gom., *Cryptomonas* sp. and *Melosira* sp.

In Lake Trummen specific nitrogenase activity in concentrated samples did not deviate significantly from activity in unconcentrated samples when comparison was based on total cell volume of heterocystous blue-green algae (Table 2). Besides the heterocystous species listed in Table 1, Lake Trummen plankton contained on different occasions *Aphanothece clathrata* W. et G. S. West, *Microcystis aeruginosa* Kütz., *Aphanocapsa delicatissima* W. et G. S. West, *Pediastrum* spp., *Scenedesmus* spp., *Staurastrum* spp., *Melosira* spp. and *Synedra* spp. (Cronberg, 1982).

In a separate experiment in Lake Bysjön with a population of *Anabaenopsis arnoldii* Aptekarj, increasing the acetylene addition to unconcentrated samples from 1.0 to 2.0 ml (after evacuation of 1.0 ml air) resulted in an increase in specific nitrogenase activity after 2 h *in-situ* incubation (Table 3). In contrast, there was no significant difference between concentrated samples receiving 1.0 and 2.0 ml acetylene ($P > 0.05$), and concentration resulted in considerably lowered specific activity.

TABLE 3. Mean specific nitrogenase activity in unconcentrated and concentrated plankton samples from Lake Bysjön after 1.0 and 2.0 ml acetylene additions*

Sample	nmol $\text{C}_2\text{H}_4 \text{ mm}^{-3} 2 \text{ h}^{-1}$	
	1.0 ml	2.0 ml
Unconcentrated	110	230
Concentrated	28	20

* At least 400 colonies and/or cells counted. Concentrations of *Anabaenopsis arnoldii*, which made up c. 80% of total phytoplankton volume, were 25 and 1000 $\text{mm}^3 \text{ l}^{-1}$ in unconcentrated and concentrated samples, respectively.

TABLE 4. pH in unconcentrated and concentrated phytoplankton samples incubated *in situ* during 2 h at 0.2 m water depth. Mean of two samples

Sample	Lake Bysjön,* 12 Aug. 1975	Lake Väckjösjön,* 27 Aug. 1975	Lake Trummen, 19 Sept. 1974
Initial	9.9	9.6	8.2
Final†			
Unconc.	9.8	9.6	8.1
Conc. 10 × ‡	9.8	10.7	—
Conc. 25 ×	10.2	10.7	—
Conc. 50 ×	10.6	10.7	—
Conc. 100 ×	10.8	10.6	10.4
Conc. 200 ×	—	—	10.7

— Not measured.

* Series I; identical to series II.

† pH measured 5–20 min after incubation; samples kept in the shade before and during measurements.

‡ Volumetric concentration factor.

Changes in pH and dissolved inorganic carbon during incubation of unconcentrated and concentrated plankton samples

In samples from Lake Bysjön concentrated more than 10 times, pH increased during incubation; higher plankton concentrations gave higher pH (Table 4). Similar effects were

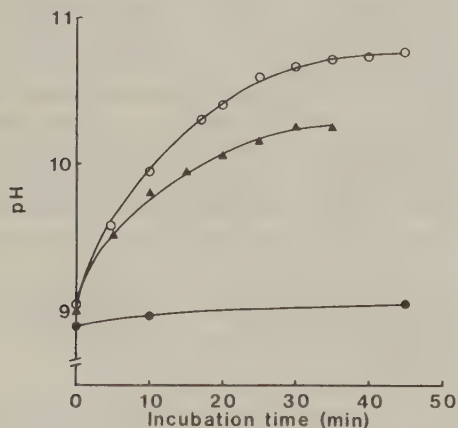


FIG. 4. pH in unconcentrated ($23 \text{ mm}^3 \text{ l}^{-1}$; ●) and concentrated ($500 \text{ mm}^3 \text{ l}^{-1}$; ▲, ○) plankton suspensions from Lake Väckjösjön on 6 September 1975. Samples were incubated in light and gently agitated every 2.5 min. Measurements started at 10.35 hours (▲), 11.55 hours (○) and 12.45 hours (●). The phytoplankton community was composed of *Anabaena spiroides* var. *spiroides* (c. 80%), *Microcystis aeruginosa* and *Raphidiopsis mediterranea* Skuja. Curves fitted by eye.

found in Lake Trummen, while in Lake Väckjösjön final pH was 10.6–10.7 in all concentrated samples. pH increased slowly in unconcentrated plankton suspensions from Lake Väckjösjön incubated in the light. In concentrated samples a pH plateau was reached in less than an hour (Fig. 4).

Changes in dissolved inorganic carbon were calculated from pH (Table 4), and from alkalinity of unconcentrated samples (Lake Bysjön 1.96, Lake Väckjösjön 0.54, Lake Trummen 0.40 meq l^{-1}). In unconcentrated samples no or small changes were found in dissolved inorganic carbon fractions during incubations, while in concentrated samples dissolved inorganic carbon decreased, and equilibria were displaced towards CO_3^{2-} . Maximal reductions in dissolved inorganic carbon during 2 h were from 1500 to $900 \mu\text{mol l}^{-1}$ in Lake Bysjön, from 450 to $110\text{--}150 \mu\text{mol l}^{-1}$ in Lake Väckjösjön, and from 400 to $30 \mu\text{mol l}^{-1}$ in Lake Trummen.

Mean inorganic carbon uptake during 2 h incubations was estimated to $3.0 \mu\text{mol mm}^{-3}$ cell volume in Lake Trummen samples concentrated 100 times, $3.6\text{--}0.3 \mu\text{mol mm}^{-3}$ in Lake Väckjösjön samples concentrated 10–100 times, and $0.6 \mu\text{mol mm}^{-3}$ in Lake Bysjön samples concentrated 25–100 times. Gelin (unpubl.) and Coveney (unpubl.) using ^{14}C -productivity techniques found gross uptake rates of $2.7 \mu\text{mol mm}^{-3} \text{ 2 h}^{-1}$ in unconcentrated surface samples from Lake Trummen on 19 September 1974, and $1.5 \mu\text{mol mm}^{-3} \text{ 2 h}^{-1}$ in unconcentrated surface samples from Lake Bysjön on 12 August 1975, respectively.

Discussion

The use of volumetric concentration factors to estimate natural nitrogenase activity by incubation of concentrated phytoplankton samples assumes that retention of nitrogen-fixing organisms by nets or filters is total. However, retention on a $10 \mu\text{m}$ net was found to be less than 100%, with a few exceptions (Fig. 1, Tables 1 and 2), and a $45 \mu\text{m}$ net gave even lower values. These results indicate that most values for nitrogenase activity in the literature based on volumetric concentration factors are underestimates. As retention varies with both sampling occasion and lake, no single correction factor can be employed. Thus, the use of

volumetric concentration factors should be avoided.

Concentration factors based on cell counts should give better estimates of natural specific nitrogenase activity in concentrated samples. However, retention of total cell volume differed greatly for different heterocystous species (Table 1) due to variation in shape and size of colonies. This resulted in changed phytoplankton community structure after concentration of multi-species samples. Consequently, nitrogenase activity of concentrated samples depended upon whether nitrogen-fixing species with high specific nitrogenase activity were retained to a greater or lesser extent than species with low specific activity.

Concentration factors based on heterocyst number and heterocyst volume were lower than concentration factors based on total cell volume of heterocystous blue-green algae (Table 2). As ultrasonic treatment of the samples was necessary to obtain random distribution of heterocysts in the counting chamber, it is probable that the difference resulted from difficulties in identifying detached heterocysts in concentrated samples rather than a true loss of heterocysts during the concentration procedure. This is supported by the fact that specific nitrogenase activity based on total cell volume was in no case significantly lower in concentrated than in unconcentrated samples from Lake Trummen, despite the apparent loss of heterocysts. This constant specific activity would otherwise have to be explained by a stimulating effect of the concentration procedure or retention by the plankton net of heterocysts with highest nitrogenase activity. Concentration factors based on heterocyst number and heterocyst volume were not significantly different, but indicate that large heterocysts might be more efficiently retained by the net or easier to count than smaller ones (Table 2). Thus, cell concentration factors, although preferable to volumetric ones, are also inadequate, at least in work with multi-species communities.

The varying effects of concentration on specific nitrogenase activity confirm the conflicting results of Stewart *et al.* (1967, 1971) and Brattberg (1977). While the decreases in specific nitrogenase activity in concentrated samples from Lake Bysjön and series II from Lake Väcksjön (Figs. 2 and 3) were effects of

concentration, similar decreases in specific rates in Lake Trummen experiments might have been masked by differential retention of vegetative cells or heterocysts in the multi-species blue-green algal communities (Tables 1 and 2). However, this combination of effects cannot explain the constant specific nitrogenase rates in unconcentrated and concentrated samples of series I from Lake Väcksjön, because only one heterocystous species was present.

Reduced nitrogenase activity in concentrated samples was not caused by insufficient substrate (C_2H_2). Acetylene concentration was about 85% of the nitrogenase saturation level (Flett *et al.*, 1976), which obviously influenced results from unconcentrated samples. However, the low specific nitrogenase activity of concentrated samples did not increase when the acetylene addition was doubled (Table 3). Decreases in dissolved acetylene during incubation were slight (0.10–2.2%) and cannot explain the large concentration effects obtained in Lakes Bysjön and Väcksjön.

Decreasing metabolic rates in concentrated organism samples has been shown for other processes than nitrogen fixation (Pomeroy & Johannes, 1968; Morris & Yentsch, 1972; Holm-Hansen, Packard & Pomeroy, 1970; Turner, 1978). In this study several factors, including physiological state of algae, were considered as possible causes of the decreasing specific nitrogenase activity in concentrated plankton samples. Since the importance of single factors, as well as their interrelationships, varies among phytoplankton communities, it is impossible to establish a common explanation for the effects on nitrogenase activity of concentrating samples from the studied lakes. I suggest the following factors as most important.

Mechanical breakage of filaments at connections between vegetative cells and heterocysts (Weare & Benemann, 1973; Paerl, 1978) may have influenced the flow of metabolites to and from heterocysts (Wolk, 1968; Weare & Benemann, 1973) and caused increased diffusion of oxygen into heterocysts. Moreover, in Lake Bysjön the concentration procedure resulted in separation of bundled trichomes of *Aphanizomenon flos-aquae*. The ecological significance of bundle formation in *A. flos-aquae* is not known, but it cannot be excluded that nitrogenase in the middle of bundles is protected from oxygen inactivation as in some

marine *Oscillatoria* (Fogg, 1974; Carpenter & Price, 1976; Bryceson & Fay, 1981).

Planktonic organisms change their environment through metabolic activity. This is especially pronounced in concentrated organism suspensions in closed experimental vessels. Changes may include pH, dissolved inorganic carbon, oxygen, nutrients, etc.

Although dissolved inorganic carbon decreased in concentrated samples, inorganic carbon availability apparently did not limit photosynthesis in Lakes Bysjön and Trummen. For Lake Trummen this was supported by comparison with ^{14}C -productivity data showing that carbon uptake was not affected by volumetric concentration of 100 times. In the well buffered Lake Bysjön, $240\ \mu\text{mol HCO}_3^- \text{ l}^{-1}$ remained at the end of the experiments even in the most concentrated samples, and concentrated samples probably did not reach a limiting pH level, since pH increased with increasing phytoplankton cell volume. In concentrated samples from Lake Väcksjön it is possible that carbon uptake rates were limited by low concentration of HCO_3^- , or by high pH *per se*, since the same pH level was reached in all levels of concentrated samples (Table 4).

Photosynthetic oxygen evolution during 2 h incubation of concentrated samples, estimated from carbon uptake rates assuming a $\text{CO}_2:\text{O}_2$ relationship of 1:1, amounted to 7–19 mg $\text{O}_2 \text{ l}^{-1}$ for Lake Bysjön, and 8–10 mg $\text{O}_2 \text{ l}^{-1}$ for both Lakes Trummen and Väcksjön. High oxygen concentrations have been shown to inhibit nitrogenase activity both in the dark and the light (Weare & Benemann, 1973). Combined with low CO_2 availability, high oxygen levels are also a prerequisite for photorespiration, which may inhibit nitrogen fixation by competition for metabolic reductant (Lex, Silvester & Stewart, 1972).

Subsaturating light conditions in concentrated samples may also have influenced nitrogenase activity (cf. Fay, 1976). Reduced nitrogenase activity was found in *in-situ* experiments when concentrated algae were exposed at twice the Secchi transparency depth or deeper in the three lakes (not shown).

In series I from Lake Väcksjön and experiments in Lake Trummen specific nitrogenase activity did not decrease in concentrated samples. To explain this it must be assumed that *Anabaena spiroides* in Lake Väcksjön was

less sensitive to environmental changes and/or photorespiration earlier in the day, or that photoinhibition in less concentrated samples counterbalanced negative effects (cf. Harris, 1978). In Lake Trummen either there were no negative effects of the concentration procedure by the same reasons as suggested for Lake Väcksjön, or changed specific nitrogenase rates were masked by differential retention of vegetative cells or heterocysts during concentration.

In conclusion, phytoplankton should not be concentrated prior to acetylene-reduction assay for nitrogenase activity. This recommendation is based on the following results:

1. Since heterocystous phytoplankton are not quantitatively retained on a $10\ \mu\text{m}$ net, use of volumetric concentration factors underestimates natural specific nitrogenase activity.

2. Use of cell concentration factors can give erroneous estimates of natural nitrogenase activity since the relative densities of nitrogen-fixing species may change during concentration.

3. In many cases concentration of phytoplankton leads to decreased specific nitrogenase activity. As several environmental factors appear to influence the extent of this decrease, it is impossible to introduce reliable correction factors.

Acknowledgments

I thank Professor S. Björk and Drs M. F. Coveney and A. Södergren for valuable criticism of the manuscript. Miss A. Fritzon did the phytoplankton analyses, Mr J. Jonasson helped with field work and GC analyses, and Mr T. Persson gave statistical advice. Dr G. Cronberg kindly supplied unpublished data on phytoplankton biomass. Financial support was given by The National Swedish Environment Protection Board, The Royal Swedish Academy of Science and The Royal Physiographical Society at Lund.

References

- Ahmad M.H. (1981) Nitrogen fixation (acetylene reduction) in a Danish lake. *Environmental Pollution* (Series A), **24**, 167–175.
- Ashton P.J. (1979) Nitrogen fixation in a nitrogen-limited impoundment. *Journal of Water Pollution Control Federation*, **51**, 570–579.

- Bengtsson L. & Gelin C. (1975) Artificial aeration and suction dredging methods for controlling water quality. *Proceedings of a Symposium on the Effects of Storage on Water Quality, Reading University, March 1975*, pp. 313–342. The Water Research Centre, Medmenham.
- Brattberg G. (1977) Nitrogen fixation in a polluted brackish water archipelago. *Ambio Special Report*, **5**, 27–42.
- Brooks R.H., Jr, Brezonik P.L., Putnam H.D. & Keirn M.A. (1971) Nitrogen fixation in an estuarine environment: the Waccasassa on the Florida gulf coast. *Limnology and Oceanography*, **16**, 701–710.
- Bryceson I. & Fay P. (1981) Nitrogen fixation in *Oscillatoria* (*Trichodesmium*) *erythraea* in relation to bundle formation and trichome differentiation. *Marine Biology*, **61**, 159–166.
- Buch K. (1945) Carbon equilibrium in the Baltic Sea. *Fennica*, **68:5** (in Swedish).
- Burris R.H. (1972) Measurement of biological N_2 fixation with $^{15}N_2$ and acetylene. *IBP Handbook No. 23: Techniques for the Assessment of Microbial Production and Decomposition in Fresh Waters* (Eds Y. I. Sorokin and H. Kadota), pp. 3–14. Blackwell Scientific Publications, Oxford.
- Carpenter E.J. & Price C.C. (1976) Marine *Oscillatoria* (*Trichodesmium*): explanation for aerobic nitrogen fixation without heterocysts. *Science*, **191**, 1278–1280.
- Cronberg G. (1982) Phytoplankton changes in Lake Trummen induced by restoration. Long-term whole-lake studies and food-web experiments. *Folia Limnologica Scandinavica*, **18**, 119 pp.
- Enell M. (1980) The phosphorus economy of a hypertrophic seepage lake in Scania, South Sweden. Dissertation, Institute of Limnology, Lund. ISSN 0348–0798, LUNBDS/(NBLI–1006)/1–190(1980).
- Fay P. (1976) Factors influencing dark nitrogen fixation in a blue-green alga. *Applied and Environmental Microbiology*, **31**, 376–379.
- Findley D.L., Findley D.I. & Stein J.R. (1973) Surface nitrogen and plankton in Skaha Lake, British Columbia (Canada). *Freshwater Biology*, **3**, 111–122.
- Flett R.J., Hamilton R.D. & Campbell N.E.R. (1976) Aquatic acetylene-reduction techniques: solutions to several problems. *Canadian Journal of Microbiology*, **22**, 43–51.
- Fogg G.E. (1974) Nitrogen fixation. *Algal Physiology and Biochemistry* (Ed. W. D. P. Stewart), pp. 560–582. Blackwell Scientific Publications, Oxford.
- Gelin C. (1975) Nutrients, biomass and primary productivity of nanoplankton in eutrophic Lake Vombsjön, Sweden. *Oikos*, **26**, 121–139.
- Gelin C. & Ripl W. (1978) Nutrient decrease and response of various phytoplankton size fractions following the restoration of Lake Trummen, Sweden. *Archiv für Hydrobiologie*, **81**, 339–367.
- Golterman H.L., Clymo R.S. & Ohnstad M.A.M. (1978) *Methods for Physical and Chemical Analysis of Fresh Waters*, 2nd edn., *IBP Handbook No. 8*. Blackwell Scientific Publications, Oxford.
- Granhall U & Lundgren A. (1971) Nitrogen fixation in Lake Erken. *Limnology and Oceanography*, **16**, 711–719.
- Hardy R.W.F., Burns R.C. & Holsten R.D. (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry*, **5**, 47–81.
- Harris G.P. (1978) Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. *Archiv für Hydrobiologie, Beiheft Ergebnisse der Limnologie*, **10**, 1–171.
- Holm-Hansen O., Packard T.T. & Pomeroy L.R. (1970) Efficiency of the reverse-flow filter technique for concentration of particulate matter. *Limnology and Oceanography*, **15**, 832–835.
- Horne A.J. & Goldman C.R. (1972) Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. *Limnology and Oceanography*, **17**, 678–692.
- Lean D.R.S., Liao C.F.-H., Murphy T.P. & Painter D.S. (1978) The importance of nitrogen fixation in lakes. *Ecological Bulletins (Stockholm)*, **26**, 41–51.
- Leonardson L. (1980) Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water. *Canadian Journal of Microbiology*, **26**, 599–605.
- Lex M., Silvester W.B. & Stewart W.D.P. (1972) Photorespiration and nitrogenase activity in the blue-green alga, *Anabaena cylindrica*. *Proceedings of the Royal Society B*, **180**, 87–102.
- Lund J.W.G., Kipling C. & Le Cren E.D. (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, **11**, 143–170.
- Morris I. & Yentsch C.S. (1972) A new method for concentrating phytoplankton by filtration with continuous stirring. *Limnology and Oceanography*, **17**, 490–493.
- Paerl H.W. (1978) Role of heterotrophic bacteria in promoting N_2 fixation by *Anabaena* in aquatic habitats. *Microbial Ecology*, **4**, 215–231.
- Pomeroy L.R. & Johannes R.E. (1968) Occurrence and respiration of ultraplankton in the upper 500 meters of the ocean. *Deep-Sea Research*, **15**, 381–391.
- Rusness D. & Burris R.H. (1970) Acetylene reduction (nitrogen fixation) in Wisconsin lakes. *Limnology and Oceanography*, **15**, 808–813.
- Stewart W.D.P., Fitzgerald G.P. & Burris R.H. (1967) *In situ* studies on N_2 fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences of the United States of America*, **58**, 2071–2078.
- Stewart W.D.P., Mague T., Fitzgerald G.P. & Burris R.H. (1971) Nitrogenase activity in Wisconsin lakes of differing degrees of eutrophication. *New Phytologist*, **70**, 497–509.
- Torrey M.S. & Lee G.F. (1976) Nitrogen fixation in Lake Mendota, Madison, Wisconsin. *Limnology and Oceanography*, **21**, 365–378.
- Turner R.E. (1978) Variability of reverse-flow concentration technique of measuring plankton respiration. *Estuaries*, **1**, 65–68.
- Utermöhl H (1958) Zur Vervollkommnung der quan-

- titativen Phytoplankton-Methodik. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **9**.
- Vanderhoef L.N. (1976) Nitrogen fixation in Lake Mendota, 1971–1973. *Hydrobiologia*, **49**, 53–57.
- Weare N.M. & Benemann J.R. (1973) Nitrogen fixation by *Anabaena cylindrica*. I. Localization of nitrogen fixation in the heterocysts. *Archiv für Mikrobiologie*, **90**, 323–332.
- Wolk C.P. (1968) Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. *Journal of Bacteriology*, **96**, 2138–2143.

(Manuscript accepted 18 October 1982)

Effects of sewage diversion in Lake Södra Bergundasjön. II. Phytoplankton changes and the role of nitrogen fixation

LARS LEONARDSON and LARS BENGTSSON

With 1 figure and 2 tables in the text

Introduction

In studies of recovery of lakes after decreased nutrient loading, the importance of phosphorus has been emphasized (AHLGREN 1973, 1977; VOLLENWEIDER & DILLON 1974; BENGTSSON et al. 1975; VOLLENWEIDER 1976). However, there is an increasing number of reports of nitrogen limiting the phytoplankton production in eutrophic lakes (CLAES-SON & RYDING 1975; FORSBERG 1975). When combined inorganic nitrogen (NO_3 , NO_2 , NH_4) is limiting to phytoplankton populations, N_2 fixation can supply new N to the ecosystem. In Lake Washington (EDMONDSON 1972), Lake Norrviken (AHLGREN 1973) and Lake Ryssbysjön (RYDING & FORSBERG 1977) summer concentrations of nitrate and ammonia were low after sewage diversion, and N_2 fixation by blue-green algae was assumed to occur. However, N_2 fixation was not measured. The aim of this study was to evaluate the significance of N_2 fixation in maintaining phytoplankton blooms after sewage diversion.

Description of the lake

Lake Södra Bergundasjön, situated in the south Swedish uplands, was the recipient of wastewater from the city of Växjö from 1927 to 1974. During this period the lake water and sediment were seriously affected by sewage effluent. To eliminate the negative effects of pollution (e. g. oxygen deficiency, fish kills, blue-green algal blooms), almost all wastewater was diverted from the lake in April, 1974. For further details of the lake and the sewage diversion, cf. BENGTSSON (1975, 1978).

Methods

Lake water was sampled in a vertical profile at the main sampling site. Methods for chemical analyses, water and nutrient budget calculations were described by BENGTSSON (1978).

Phytoplankton primary production was measured monthly during April to September with the ^{14}C method (STEEMANN NIELSEN 1952, 1965). Bottles were exposed in a vertical profile at 5–7 depths (0.1–1.5 m) at the main sampling site from noon to sunset. A well-mixed water sample from 0–1 m was used for all bottles and for the determination of phytoplankton biomass.

Phytoplankton fresh weight (F. W.) was determined with the UTERMÖHL technique (cf. WILLÉN 1976). Blue-green algae were counted after ultrasound treatment.

Nitrogen fixation was determined with the acetylene reduction technique (STEWART et al. 1967). Sampling and exposure were usually done at four depths at the main sampling site. On six occasions during 1974–1976 when N_2 fixation was low, a mixed water sample from the layer 0–1 m was incubated at 0.2 m and the areal fixation estimated. In 1974 the phytoplankton were concentrated 80–200 times with plankton nets (mesh 10 μm) before exposure. In 1975 and 1976 unconcentrated samples were

used. 5 ml lake water was incubated in 15 ml test tubes for two hours around noon after injection of 1.0 ml C_2H_2 . 0.15 ml LUGOL's solution (WILLÉN 1962) was used to stop the acetylene reduction process. All incubations were done in triplicate, and blanks fixed with LUGOL's solution before C_2H_2 addition were included.

Ethylene was determined by gas chromatography with a flame ionization detector (Varian Aerograph, series 1520) using a 2.7 m stainless steel column ($1/16''$) packed with Porapak T. The temperature of the column oven was 90 °C. N_2 was used as carrier gas at a flow rate of 30 ml/min. Daily rates of acetylene reduction were calculated on the basis of two diurnal studies in August, 1973, in the adjacent Lake Trummen. Seasonal rates were estimated by integrating the daily rates over the study period. Values were corrected for the solubility of C_2H_4 in the liquid phase. To convert C_2H_4 to NH_4 , the theoretical molar ratio of 1.5 : 1 was used (BERGERSEN 1970).

Results

Nutrient concentrations

The reduced input of nutrients to Lake Södra Bergundasjön after sewage diversion resulted in decreased mean annual concentrations of total N and total P, calculated for the whole water mass. Total N decreased from 4.8 and 4.4 mg N/l in 1973 and 1974, respectively, to 3.1 and 3.6 mg N/l in 1975 and 1976, and total P from 1.2 and 1.3 mg P/l to 0.8 and 0.8 mg P/l, respectively ($n = 11-14$). Combined inorganic N concentrations were high during the winter months and lower during the spring and summer (Fig. 1 A). Before sewage diversion the lowest inorganic N concentration in the surface water was 0.21 mg N/l, while the lowest concentrations during 1974-1976 were in the range 0.01-0.04 mg N/l. Contrary to combined inorganic N, phosphate concentrations were maximal during late summer and autumn. This was due to a high net sediment release (cf. BENTSSON 1978) in combination with usually mixed conditions in the lake. Phosphate spring minima were not below 0.12 mg P/l during the study period.

Phytoplankton

In 1973 and 1974 maximum phytoplankton biomass (52 and 73 mg F.W./l, respectively) was recorded when concentrations of phosphate and combined inorganic N were high, while in 1975 and 1976 the maxima (27 and 57 mg F.W./l, respectively) appeared when combined inorganic N was almost depleted. Phytoplankton biomass development was well correlated with primary production, and maxima coincided with transparency readings of less than 0.5 m (Fig. 1 B-C). In the summer of 1974 the lake was still influenced by the sewage effluent that was discharged until the end of April. The low biomass in 1975 probably was a combined effect of sewage diversion and low nutrient import from the rest of the drainage area during the spring (BENTSSON 1978).

Although no significant changes in the weighted means of SECCHI disc transparency, phytoplankton biomass and primary production were noted during June to September 1973-1976 (Table 1), summer phytoplankton species composition changed drastically after the sewage diversion. During 1973 and 1974 *Microcystis* spp. (especially *M. aeruginosa* KÜTZ. and *M. viridis* (A. BR.) LEMM.) accounted for as much as 95 % of the total summer phytoplankton biomass and

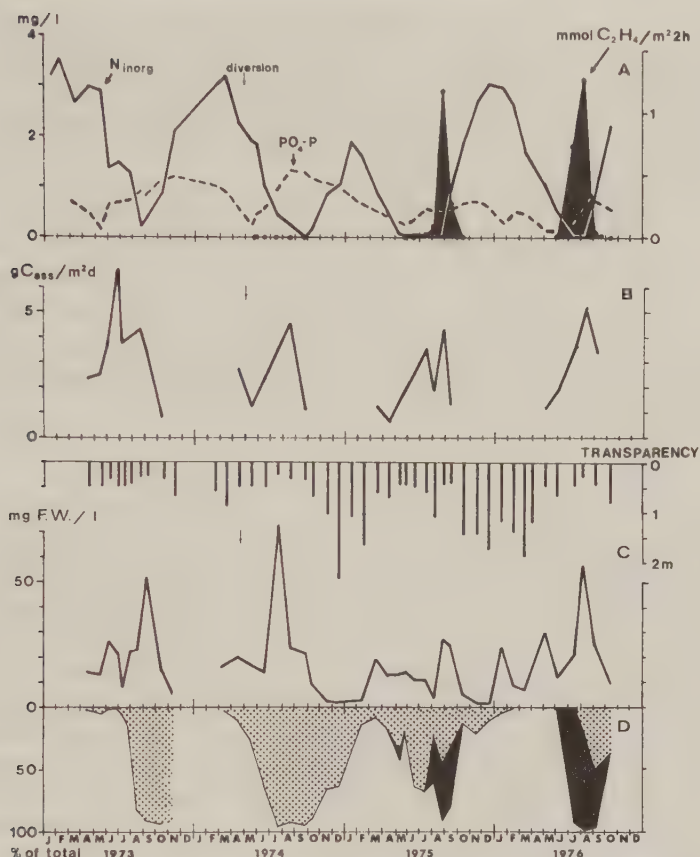


Fig. 1. Changes in nutrient concentrations and phytoplankton in Lake Södra Bergunda-sjön, 1973—1976. A: combined inorganic nitrogen (solid line) and dissolved inorganic phosphorus (broken line) at 1 m, and ethylene production (black). B: assimilated carbon. C: SECCHI disc transparency (above) and phytoplankton fresh weight biomass (0—1 m). D: phytoplankton biomass composition (percent): N_2 -fixing blue-greens (black), *Microcystis* (dotted) and non blue-greens (white). Arrows indicate the start of sewage diversion.

formed nuisance blooms. In 1975 and 1976 N_2 -fixing blue-green algae increased significantly and became dominant in the algal blooms. In May, 1975, *Aphanizomenon flos-aquae* RALFS appeared in low numbers (0.15 mg F.W./l). In August and September of the same year *Anabaena spiroides* f. *crassa* (LEMM.) ELENK. developed in concentrations up to 13 mg F.W./l, corresponding to about 50 % of the total phytoplankton biomass. *Microcystis* species represented 24—41 % of the total biomass at the same time. In summer 1976 *Anabaena spiroides* f. *crassa* appeared in June and reached a maximum in early August (44 mg F.W./l, or 78 % of the total phytoplankton biomass). In autumn the dominating *Anabaena* was succeeded by *Microcystis* species.

Besides the blue-green algae, other phytoplankton species, e. g. *Scenedesmus* spp., *Pediastrum boryanum* (TURP.) MENECH., *Cryptomonas* spp., *Melosira* spp.,

Table 1. SECCHI disc transparency, phytoplankton biomass and primary production in Lake Södra Bergundasjön. Weighted means during June to September, 1973—1976.

	1973 ¹	1974 ²	1975 ³	1976 ²
Transparency, m	0.37	0.35	0.55	0.47
Phytoplankton				
biomass, mg F.W./l	25	30	16	27
production, g C/m ²	485	360	315	460

¹ n = 6, ² n = 4, ³ n = 5.

Asterionella formosa HASS. and *Stephanodiscus hantzschii* GRUN., were found from June to September. However, compared to the blue-green algae their biomass was of minor importance.

Nitrogen fixation

As concentrations of combined inorganic N were high (≥ 0.21 mg N/l) and only occasional filaments of a N₂-fixing species (*Anabaena spiroides* f. *crassa*, < 0.01 mg F.W./l) were found, it is assumed that N₂ fixation was of no significance during 1973 (Fig. 1 A, D). In 1974 no N₂-fixing species were observed and no N₂ fixation was detected. In May, 1975, there was a transitory appearance of *Aphanizomenon flos-aquae*, but it was not until 1.5 months later after the decrease in nitrate and ammonia concentrations (to 0.02—0.05 mg N/l) that the *Anabaena* bloom and substantial N₂ fixation were recorded. In 1976 the depletion of combined inorganic nitrogen (to 0.04 mg N/l) was immediately followed by an increase in the biomass of *Anabaena*, and intensive N₂ fixation was measured. Maximum acetylene reduction rates recorded during 1975—1976 amounted to about 1.2 and 1.3 mmol C₂H₄/m² · 2h. This corresponds to N₂ fixation rates of c. 60 and 65 mg N/m² · day. The annual N inputs by N₂ fixation were estimated to 1.6 g N/m² in 1975 and 2.9 g N/m² in 1976. Over 96 % of this fixation occurred during June—September (Table 2).

Table 2. Nitrogen inputs to Lake Södra Bergundasjön during the growing season (June—September) 1973—1976 (g N/m²; — = not measured).

	1973	1974 ¹	1975	1976
From treatment plant and storm water	5.7	0.3	1.0	0.3
From rest of drainage area	0.3	0.0	0.3	0.3
From precipitation	0.1	0.2	0.2	0.1
From N ₂ fixation	—	0.0	1.6	2.9
Total input	6.1	0.6	3.1	3.5
N ₂ fixation as % of total input	—	0	51	82

¹ Sewage diversion in April 1974.

Discussion

In Lake Södra Bergundasjön neither N nor P limited phytoplankton growth in 1973 and 1974 (Fig. 1 A). After sewage diversion in 1974 combined inorganic N became growth limiting during the summers, and there was a drastic change from an almost total dominance of *Microcystis* to increasing populations of N₂-fixing species of *Anabaena* and *Aphanizomenon* (Fig. 1 D). Thus, the phytoplankton community adapted to the changed nutrient situation, and the ecosystem was compensated for the decreased N input. Similar observations on phytoplankton development after sewage diversion were reported for Lake Washington (EDMONDSON 1972) and Lake Norrviken (AHLGREN 1973).

N₂ fixation made up c. 13 and 30 %, respectively, of the annual nitrogen input to Lake Södra Bergundasjön during 1975 and 1976 (cf. BENGTSSON 1978). The importance of N₂ fixation in supplying the primary producers with N is even more evident when the comparison is confined to the period June—September 1973—1976 (Table 2). N₂ fixation was 51 and 82 %, respectively, of total N input during the growing season in 1975 and 1976, i. e. the N supply was more than doubled by biological activity. In 1974 there was no N₂ fixation, as summer concentrations of combined inorganic N in the lake were still influenced by the spring sewage discharge (Fig. 1 A). To better understand the role of fixed N₂ in the lake ecosystem it is necessary to investigate N exchange between organisms, sediment release and deposition, and denitrification.

The importance of high P availability to N₂-fixing algae was discussed by several authors (STEWART & ALEXANDER 1971; HORNE et al. 1972; SCHINDLER 1974; LUNDGREN 1978). In Lake Södra Bergundasjön phosphate concentrations were high at the beginning of the summer (Fig. 1 A) and increased further due to phosphorus release from the sediment (BENGTSSON 1978). These necessary prerequisites, together with N₂ fixation, maintained high biomass of blue-green algae after the sewage diversion.

Contrary to our findings for Lake Södra Bergundasjön, MICHALSKI & CONROY (1973) reported that discontinuing the industrial P discharge into Little Otter Lake resulted in a change from eutrophic to oligotrophic conditions. At the same time the high biomass of *Anabaena limnetica*, a potential N₂-fixing species, was greatly reduced. The differences in the reactions of Little Otter Lake and Lake Södra Bergundasjön to sewage diversion might be explained by two facts. The short pollution period in Little Otter Lake may have resulted in a low P deposition in the sediment and, as a consequence, insignificant P release from the sediment after sewage diversion. Furthermore, a new nutrient equilibrium (total P < 20 µg/l) was rapidly achieved in Little Otter Lake as the water renewal time was extremely short, on average 1 month.

The experiences discussed above indicate that as long as there is an excess of available P and other nutrients in recipients, it is of no use to build advanced treatment plants for N removal. Furthermore, the diversion of sewage effluents from polluted shallow recipients is not sufficient in the short run to avoid plankton blooms unless release of P from the sediment can be repressed or the water renewal time is very short.

Acknowledgements

The authors thank Miss A. FRITZON for carrying out the plankton analyses, Dr. A. SÖDERGREN and Mr. W. RIPL for valuable criticism of the manuscript and Mr. M. F. COVENEY for the linguistic revision. The National Swedish Environment Protection Board financed the investigation.

References

- AHLGREN, I., 1973: Limnologiska studier av sjön Norrviken. III. Avlastningens effekter. — *Scripta Limnologica Upsaliensia* 333.
- 1977: Role of sediments in the process of recovery of a eutrophicated lake. — *Interactions between sediments and fresh water*: 372—377. Proceedings of an international symposium held at Amsterdam, the Netherlands, September 6—10, 1976.
- BENGTTSSON, L., 1975: Phosphorus release from a highly eutrophic lake sediment. — *Verh. Internat. Verein. Limnol.* 19: 1107—1116.
- 1978: Effects of sewage diversion in Lake Södra Bergundasjön. I. Nitrogen and phosphorus budgets. — *Vatten* (1): 2—9.
- BENGTTSSON, L., FLEISCHER, S., LINDMARK, G. & RIPL, W., 1975: Lake Trummen restoration project. I. Water and sediment chemistry. — *Verh. Internat. Verein. Limnol.* 19: 1080—1087.
- BERGERSEN, F. J., 1970: The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. — *Aust. J. Biol. Sci.* 23: 1015—1025.
- CLAESSON, A. & RYDING, S.-O., 1975: Nitrogen — a growth limiting nutrient in eutrophic lakes. — Conference on nitrogen as a water pollutant. IAWPR specialized conference, Copenhagen, Denmark, 18—20th August 1975.
- EDMONDSON, W. T., 1972: The present condition of Lake Washington. — *Verh. Internat. Verein. Limnol.* 18: 284—291.
- FORSBERG, C., 1975: Nitrogen as a growth factor in fresh water. — Conference on nitrogen as a water pollutant. IAWPR specialized conference, Copenhagen, Denmark, 18—20th August 1975.
- HORNE, A. J., DILLARD, J. E., FUJITA, D. K. & GOLDMAN, C. R., 1972: Nitrogen fixation in Clear Lake, California. II. Synoptic studies on the autumn *Anabaena* bloom. — *Limnol. Oceanogr.* 17: 693—703.
- LUNDGREN, A., 1978: Nitrogen fixation induced by phosphorus fertilization of a sub-arctic lake. — *Ecol. Bull.* (Stockholm) 26: 52—59.
- MICHALSKI, M. F. P. & CONROY, N., 1973: The "oligotrophication" of Little Otter Lake, Parry Sound District. — *Proc. 16th Conf. Great Lakes Res.*: 934—948. Internat. Assoc. Great Lakes Res.
- RYDING, S.-O. & FORSBERG, C., 1977: Sediments as a nutrient source in shallow polluted lakes. — *Interactions between sediments and fresh water*: 227—234. Proceedings of an international symposium held at Amsterdam, the Netherlands, September 6—10, 1976.
- SCHINDLER, D. W., 1975: Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. — *Verh. Internat. Verein. Limnol.* 19: 3221—3231.
- STEEMANN NIELSEN, E., 1952: The use of radioactive carbon (C^{14}) for measuring organic production in the sea. — *J. Cons. perm. int. Explor. Mer* 18: 117—140.
- 1965: On the determination of the activity in ^{14}C -ampoules for measuring primary production. — *Limnol. Oceanogr. Suppl.* 10: 247—252.
- STEWART, W. D. P. & ALEXANDER, G., 1971: Phosphorus availability and nitrogenase activity in aquatic blue-green algae. — *Freshwat. Biol.* 1: 389—404.
- STEWART, W. D. P., FITZGERALD, G. P. & BURRIS, R. H., 1967: In situ studies on N_2 fixation using the acetylene reduction technique. — *Proc. Nat. Acad. Sci. US* 58: 2071—2078.

- VOLLENWEIDER, R. A., 1976: Rotsee, a source, not a sink for phosphorus? A comment to and a plea for nutrient balance studies. — *Schweiz. Z. Hydrol.* 38: 29—34.
- VOLLENWEIDER, R. A. & DILLON, P. J., 1974: The application of the phosphorus loading concept to eutrophication research. — Publ. of the Environmental Secretariat, NRC Associate Committee on Scientific Criteria for Environmental Quality. NRCC no 13690.
- WILLÉN, E., 1976: A simplified method of phytoplankton counting. — *Br. Phycol. J.* 11: 265—278.
- WILLÉN, T., 1962: Studies on the phytoplankton of some lakes connected with or recently isolated from the Baltic. — *Oikos* 13: 169—199.

Authors' address:

L. LEONARDSON and L. BENGSSON, Institute of Limnology, Fack, S-220 03 Lund, Sweden.

This is a reprint of

Verhandlungen - Proceedings - Travaux

of the International Association for Theoretical and Applied Limnology,
Congress in Denmark 1977

If you are a member of this association you receive these "Proceedings" against your yearly membership fee of Swiss Francs 45.- and also the

Mitteilungen - Communications

of the International Association for Theoretical and Applied Limnology that are published irregularly. The latest number was no. 21,
„Experimental Use of Algal Cultures in Limnology, a symposium“.

Please notice also the

Archiv für Hydrobiologie

an official organ of the International Association for Theoretical and Applied Limnology and its Supplements.

As a member of this association you are entitled to receive this important journal, edited by Professor Dr. H.-J. ELSTER, Konstanz, and Professor Dr. W. OHLE, Plön, at a special membership price.

True to its tradition this periodical serves freshwater research in the widest sense, including treatment of problems of brackish and seawater as far as they bear a relationship to limnology. It is the editors' aim to devote increased attention to ecology in association with experimental, above all physiological works corresponding to the more recent developments in limnology. Finally, it is intended that the "Archiv" should continue to form a bridge between theoretical and applied water research.

For details please write to the Publishers E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Johannesstraße 3 A, D-7000 Stuttgart 1.

If you are interested in

Archiv für Hydrobiologie, Supplements

and in the special issues

Ergebnisse der Limnologie

these are available also against a special membership price; for details please ask the Publishers.

If you are interested in being a member of the International Association for Theoretical and Applied Limnology, please write to the General Secretary-Treasurer:

Prof. Dr. R. G. WETZEL
W. K. Kellogg Biolog. Station
Michigan State University
Hickory Corners, Michigan 49060/U. S. A.

BLUE-GREEN ALGAL NITROGEN FIXATION IN LAKE TRUMMEN
AFTER RESTORATION

Lars Leonardson
Institute of Limnology
University of Lund
Box 3060
S- 220 03 LUND
Sweden

ABSTRACT

Nitrogen fixation (acetylene reduction) was measured during four post-restoration years in Lake Trummen, Sweden. Vertical, diurnal, seasonal and long-term aspects were studied. Two consecutive diurnal studies showed that, although symmetric insolation over the day, 60 and 69% of diurnal acetylene reduction occurred in the afternoon, while 55% of carbon fixation took place before noon. Nocturnal rates contributed c. 15% to diurnal nitrogenase activity. Seasonal patterns were regulated by concentrations of nitrate and ammonium and turnover of seston nitrogen. Late-summer declines coincided with increased levels of nitrate and ammonium, and decreasing temperature (1973), and phosphorus or iron deficiency (1974, 1976). Estimated annual rates of nitrogen fixation amounted to 3.4, 1.7, 1.9 and 0.12 g N m⁻² for 1973, 1974, 1976 and 1978. This was estimated to correspond to 24-1% of total nitrogen input to Lake Trummen. Pre-restoration nitrogen fixation was calculated to be in the range 3.4-60 g N m⁻² year⁻¹. The long-term decline in N₂ fixation was assigned to successively decreasing phosphorus availability, induced by the sediment dredging in 1970-1971. The large decrease in 1978 was suggested to be a result of large-scale fish elimination, influencing the zooplankton assemblage structure. As a consequence the biomass of Aphanothece clathrata increased. This small-sized alga did not fix dinitrogen, but most probably outcompeted heterocystous blue-greens for phosphorus, which resulted in very low nitrogen-fixation rates.

INTRODUCTION

Lake Trummen, a formerly oligotrophic lake of the South Swedish Archaean bed-rock plain (Digerfeldt 1972, and Tab. 1), was heavily eutrophicated by domestic and industrial wastewater, and urban stormwater in the beginning of the 20th century. In 1958, wastewater was diverted from the lake, but as a consequence of regeneration of nutrients from the heavily polluted sediment the lake did not recover. During this period, Lake Trummen was characterized by high nutrient concentrations, dense blue-green algal summer blooms, increasing macrophyte cover, and high sediment oxygen consumption which in winters with extensive ice-cover periods resulted in fish kills. In 1970 and 1971 Lake Trummen was restored by means of removal of the uppermost nutrient-rich organogenic sediment layer, and by macrophyte elimination (Björk 1972, Björk et al. 1972).

Investigations before, during and after restoration showed that great changes were induced in most structural and functional elements of the ecosystem (Bengtsson et al. 1975, Cronberg et al. 1975, Andersson et al. 1975). Before restoration the release of phosphorus and nitrogen from the sediment was high during summer (Bengtsson and Fleischer 1971), while after the dredging the internal loading was diminished (Bengtsson et al. 1975). The resulting lower nutrient availability in the lake water limited phytoplankton development, and mean annual phytoplankton primary production diminished from 370 g C m^{-2} during 1968-1969 to 225 g C m^{-2} during 1972-1973 (Gelin & Ripl 1978). Mean phytoplankton biomass during summer decreased with about 90% to 7 mg FW l^{-1} from 1968-1969 to 1976-1978. Summer phytoplankton assemblage composition changed from dominance of Microcystis spp., and large-sized heterocystous blue-green algae to small colony-forming blue-green algae, diatoms and undetermined micro-algae. During 1976-1978 also selective fishing of roach and bream probably contributed to the continued oligotrophication of the lake (Cronberg 1982).

No nitrogen-fixation studies were made during the pre-restoration period. Yet this process was felt to contribute considerable amounts of nitrogen to the ecosystem during summers. The conclusion was based on the occurrence of high concentrations of known nitrogen-fixing blue-green algae, e.g. Aphanizomenon flos-aquae (L.) Ralfs, Anabaena solitaria f. smithii Kom., and A. spiroides f. crassa Lemm. (Cronberg 1982), actually carrying heterocysts.

Generally, the reduced availability of dissolved inorganic nitrogen caused by the restoration should increase the competitiveness of N_2 -fixing organisms. However, the simultaneously induced lower availability of other nutrients probably should outweigh the advantages, and hamper the development of nitrogen fixers. Therefore, the purpose of this investigation was to study the changes in blue-green algal nitrogen fixation after the restoration, and relate these to changes in the lake's nutrient status. The study included examination of diurnal, vertical, seasonal and long-term development of N_2 fixation, as well as estimation of nitrogen contributions to the ecosystem via nitrogen fixation. An attempt was also made to estimate pre-restoration nitrogen fixation.

MATERIALS AND METHODS

Water chemistry

Lake water was taken with a Ruttner sampler in the middle of the lake, at 0.2 and c. 2.0 m water depth during 1968-1975, and as a mixed sample from 0-2 m during 1976-1978.

Analytical methods used were described by Gelin (1975). In phosphorus analyses cystein was used for masking mercury used for preservation of the water samples. Total iron was determined with a Perkin Elmer 303 Atomic Absorption Spectrophotometer. After 1970 nitrogen and phosphorus fractions were analyzed with a Technicon Auto Analyzer II.

Seston was sampled from 0-1 m water depth. Dry weight was determined on Whatman GF/C filters ($n \geq 4$) dried at 105 °C. Filters were stored frozen until analysis. Nitrogen was analyzed using a Carlo Erba Elemental Analyzer, model 1106. Seston phosphorus was determined according to Solorzano & Sharp (1980). Seston N:P ratios were expressed on weight basis.

Seston phosphorus did not agree with total phosphorus measurements during 1974 and 1976. Ahlgren (1976) found that the total-phosphorus method used (Menzel & Corwin 1965) on average recovered 89%, in one case only 70%, of total phosphorus in waters rich in clay and plankton, compared to analyses involving both oxidation and hydrolysis. Thus, it is probable that total phosphorus in Lake Trummen was undervaluated, and that this effect was greater when phytoplankton biomass was high. The seston-phosphorus method used recovered 100% of phosphorus in hard-decomposable organic compounds (Solorzano & Sharp 1980), and gave

linear response when applied at high concentrations ($>10\,000\text{ ug P l}^{-1}$) of adenosine-triphosphate and blue-green algae (Leonardson unpubl.).

Nitrogen fixation

Nitrogen fixation was studied with a modified acetylene-reduction technique (Stewart et al. 1967) during May-Oct in 1973, 1974, 1976 and 1978. Sampling was made at 0.2, 0.5, 1.0 and 1.5 m water depth at the main sampling site. During 1973 through July 1974 heterocystous blue-green algae were concentrated 4-100X by plankton nets (mesh openings 10 or 45 μm). Acetylene-reduction rates of concentrated samples were corrected for by the ratio of cell volume of heterocystous algae in unconcentrated samples:cell volume of heterocystous algae in concentrated samples. Concentrating phytoplankton in Lake Trummen was shown not to influence specific acetylene-reduction rates (Leonardson 1983). 5.0 ml algal suspension was pipetted into 15.0 ml test tubes closed with butyl-rubber septa and screw caps. 1.0 ml acetylene, corresponding to 0.07 ml C_2H_2 ml H_2O^{-1} (Flett et al. 1976), was added in experiments until July 1976, after which 2.0 ml acetylene was injected after evacuation of 1.0 ml air. Since at 1.0 ml acetylene the nitrogenase was not fully saturated, a correction factor of 1.4 was used (Leonardson in prep.). The experiments were carried out in triplicate. One "blank" tube containing organisms preserved with 0.15 ml Lugol's solution was incubated at each sampling level. Incubations were made in situ, usually from 1130 to 1330 hours, after which acetylene reduction was stopped by injection of 0.15 ml Lugol's solution.

Samples were agitated 30-60 s before removal of gas for analysis. 1.0 ml gas from the test tubes was analyzed for ethylene by a gas chromatograph with a flame ionization detector (Varian Aerograph, series 1520 and 3700), using a 2.7 m stainless steel column (1.6 mm inner diameter) packed with Porapak T and operated at 90°C . Nitrogen was utilized as carrier gas at a flow rate of 30 ml min^{-1} . In calculations of acetylene-reduction rates loss of ethylene from the vapour phase due to solubility and chemical reaction with Lugol's solution was considered (Leonardson 1980).

Diurnal rates of acetylene reduction were calculated on basis of two 24-h studies in Aug 1973, presented in this paper. Seasonal rates were estimated by integrating diurnal rates over the study period. To convert C_2H_4 to NH_4 , the empirically found ratio of 2.2:1 was used (Peterson & Burris 1976).

Phytoplankton biomass

Contents of incubation tubes for acetylene-reduction measurements were used for counting heterocystous blue-green algae from 3-4 depths with the inverted microscope technique (cf. Leonardson 1983). In determining the fresh weight of algae from cell volumes, algal specific weight was assumed to be 1.0. Published data on fresh weight of non-heterocystous algae were used when available (Cronberg 1982). Since vertical stratification of algae was rare in Lake Trummen (Gelin & Ripl 1978, Cronberg 1982), counts of non-heterocystous algae at 0.2 m water depth were considered to be valid for the whole water volume. Results are presented as whole-lake volume-weighted fresh weight per unit lake area.

Nitrogen budget

Water inflow to Lake Trummen was calculated from water level observations (5 days weekly) at calibrated weirs in the five largest inlet streams and in the outflow. Sampling for nitrogen was made 1-2 times per month, and weighted means used for budget calculations. Since total calculated water inflow was only c. 50% of outflow volumes during all years, it is assumed that diffuse inflow of groundwater was considerable, or that weirs were not correctly calibrated. Therefore, until weirs are controlled and recalibrated no thorough budget can be done.

Meteorological data

During the diurnal studies in 1973 irradiance was registered with a solarimeter (Kipp & Zonen, Delft, the Netherlands) equipped with a thermopile mounted under two glass domes, and placed at a high locality close to Lake Trummen. The resolution was one measurement per min. Results were integrated by The Swedish Meteorological and Hydrological Institute (SMHI). For estimations of diurnal acetylene reduction in the seasonal budget, average insolation for Malmö, Göteborg and Stockholm was used (SMHI unpubl.).

Precipitation data were derived from monthly publications of SMHI.

RESULTS

Vertical distribution of acetylene reduction

Since Lake Trummen is shallow and much exposed to wind the water is homothermal during most of the ice-free period (Fig. 1A). The greatest temperature difference registered between 0.2 and 1.5 m water depth during this investigation was 3.4°C , while most discrepancies were less than 0.5°C . Vertical gradients of pH and micro-nutrients usually were small during the vegetation periods (Fig. 1A and 2). Phosphate and nitrate rarely ($n=4$ for $\text{PO}_4\text{-P}$, $n=2$ for $\text{NO}_3\text{-N}$) showed differences greater than $5\text{ }\mu\text{g l}^{-1}$ between 0.2 and 1.5 m, while ammonium deviated more than $10\text{ }\mu\text{g N l}^{-1}$ between surface and bottom on nine occasions during the summers 1973-1975 ($n=29$).

The vertical homogeneity was reflected in the distribution of nitrogen-fixing blue-green algae (Fig. 1B). Only on seven sampling dates there were higher concentrations ($\geq 20\%$) of heterocystous algae at 0.2 or 0.5 m water depth than at deeper levels, e.g. on 16 Aug 1973 Anabaena lemmermannii P. Richt. and A. viguieri Denis et Frémy made up 3.5 mg FW l^{-1} at 0.2-0.5 m and 0.5 mg FW l^{-1} at 1.0-1.5 m, while Aphanizomenon gracile Lemm. was evenly distributed (0.8 mg FW l^{-1}).

On most occasions maximum rates of acetylene reduction were found at 0.2 or 0.5 m water depth, approximately corresponding to 50-75% of the Secchi transparency depth (Fig. 1A and 1B). Often almost identical acetylene reduction was registered on all sampling levels, and in exceptional cases profiles with double maxima were found.

Due to the even vertical distribution of the heterocystous algae, specific nitrogenase activity ($\text{mol C}_2\text{H}_4\text{ mg FW}^{-1}\text{ 2 h}^{-1}$) showed similar vertical patterns as activity expressed per unit water volume (Fig. 1C). Usually specific rates at 1.5 m water depth were higher than 40% of surface rates. During the whole study period surface inhibition of specific acetylene reduction was found on 14 occasions, and occurred both on sunny and cloudy days, while surface maxima only were found on cloudy occasions. On these days oxygen saturation at 0.2 m varied between 95 and 145%. Regression analysis of vertical gradients of specific acetylene reduction (dependent variable), and gradients of light, phosphate, nitrate, ammonia and the sum of nitrate and ammonia (independent variables) only showed a significant linear correlation between activity and phosphate gradients ($p<0.05$, $r^2=0.26$).

Diurnal variation of acetylene reduction

Diurnal studies were performed on 28 and 29 Aug 1973. Incubations were made from 0400 h to 2200 h in c. 2-h intervals at 0.2, 1.0 and 1.5 m water depth, except around noon when also 0.5 m was included.

On 28 Aug weather was clear and calm, while on the following day the sky was hazy in the morning and cloudy from noon. A slow wind was blowing on 29 Aug preventing vertical heterogeneity in physical and chemical parameters, and in phytoplankton distribution (Fig. 3). Heterocystous blue-green algae present were Anabaena lemmermannii and Aphanizomenon gracile at about 5 mg FW l^{-1} . From noon to evening on 28 Aug A. lemmermannii accumulated at 0.2 and 0.5 m water depth, which resulted in 2.7 mg FW l^{-1} at these levels, compared to 1.8 mg FW l^{-1} at 1.5 m. Since the vertical distribution of A. gracile was even (2.5 mg FW l^{-1}), the total density of heterocystous algae at the surface exceeded that of the deeper layers with only 20%.

Maximum acetylene reduction per unit water volume occurred between 1200 h and 1400 h on both days, i.e. at or immediately after maximum insolation on the lake surface (Fig. 4). Low acetylene reduction was found after sunset on both days. In order to calculate 24-h acetylene reduction, nocturnal rates were estimated to equal $0.2 \text{ mmol C}_2\text{H}_4 \text{ m}^{-2} 2 \text{ h}^{-1}$ (mean of the two evening measurements/2; Fig. 4). Whole-lake volume-weighted acetylene reduction was estimated to 11.4 and 10.3 $\text{mmol C}_2\text{H}_4 \text{ m}^{-2} 24 \text{ h}^{-1}$ on 28 and 29 Aug. The difference (10%) agreed well with the decrease in total insolation (15%) the second day. On both days c. 15% of the 24-h acetylene reduction was assigned to nocturnal activity.

Although insolation during both days was evenly distributed between morning and afternoon (50/50% and 53/47%), afternoon acetylene-reduction rates were higher than morning rates, and made up 60 and 69% of the total diurnal activities. Contributing to the noon/afternoon difference on 29 Aug was decreased surface acetylene reduction, and stagnated rates at greater water depths between 1000 h and 1200 h (Fig. 5). The reason to this is not known, but at 1115 h decreased oxygen concentrations and pH were found at 1.5 and 1.9 m water depths (Fig.3).

Contrary to acetylene-reduction, 55% of the whole-lake volume-weighted carbon fixation ($1580 \text{ mg C m}^{-2} \text{ d}^{-1}$) took place before noon on 28 Aug 1973 (Gelin pers. comm., half-day measurements only performed on 28 Aug; cf. Gelin

& Ripi 1978). The results were true for the total phytoplakton assemblage as well as for two algal size fractions separated by plankton nets with 10 μm hole side.

Daily amplitudes of acetylene-reduction rates were greater in the upper water layers than at 1.5 m (Fig. 5). Between 1000 and 1200 h on both investigation days, and after noon on 28 Aug lower activities were found at 0.2 m than at 0.5 or 1.0 m. Due to the *Anabaena* surface accumulation in the afternoon of 28 Aug, this was even more conspicuous when rates were expressed per unit fresh weight per liter.

To estimate 24-h acetylene reduction from 2-h incubations (between 1130 h and 1330 h) on other sampling dates, the ratio of daily insolation:incubation-period insolation was intended to be used. However, these ratios amounted to 4.25:1 and 3.90:1 on 28 and 29 Aug, and differed significantly from the estimated ratios of 24-h acetylene reduction:2 h incubation-period acetylene reduction, 6.45:1 and 5.68:1, respectively. Therefore, insolation ratios had to be corrected before the calculation of 24 h acetylene reduction, i.e. $6.45/4.25 = 5.68/3.90 = 1.5 = \text{correction factor}$.

Seasonal variation of acetylene reduction

Acetylene reduction was detected between May and October (Fig. 6), but activities higher than $100 \text{ nmol C}_2\text{H}_4 \text{ l}^{-1} 2 \text{ h}^{-1}$ (corresponding to $0.6 \text{ ug N l}^{-1} 2 \text{ h}^{-1}$), were limited to two months or less each year.

Maximum acetylene reduction amounted to 3.6, 0.66, and $1.6 \text{ nmol C}_2\text{H}_4 \text{ m}^{-2} 2 \text{ h}^{-1}$ in 1973, 1974, and 1976, respectively. Periods of high acetylene reduction were characterized by temperatures of $13\text{--}23^\circ\text{C}$, pH 7.5–10, low concentrations of dissolved inorganic nitrogen and phosphate ($\text{NH}_4^+ + \text{NO}_2 + \text{NO}_3\text{-N}$ $10\text{--}40 \text{ ug l}^{-1}$, and $\text{PO}_4\text{-P}$ $5\text{--}20 \text{ ug l}^{-1}$), moderately high concentrations of total iron ($0.3\text{--}1.0 \text{ mg l}^{-1}$), and presence of filamentous blue-green algae actually carrying heterocysts. In summer 1978 heterocystous blue-green algae were sparse ($\leq 0.6 \text{ mg FW l}^{-1}$), and acetylene reduction low. Maximum activity ($0.05 \text{ nmol C}_2\text{H}_4 \text{ m}^{-2} 2 \text{ h}^{-1}$) was then reached in the middle of August.

In 1973 and 1976 acetylene reduction increased immediately after the spring decrease of ammonium and nitrate, but in 1974 it did not start until about one month after these nutrients reached 20 ug N l^{-1} or less (Fig. 6). The late-summer decline in acetylene reduction in 1973 occurred simultaneously

with an increase in dissolved inorganic nitrogen and a decrease in temperature, while in 1974 and 1976 the activity decreased about one month before the increase of dissolved inorganic nitrogen. The quotient of dissolved inorganic nitrogen to phosphorus ($\text{DIN:PO}_4\text{-P}$) showed similar seasonal pattern as the sum of ammonium and nitrate. During winter it reached 100-200:1, decreased during spring, and remained at 1-5:1 during most of the nitrogen-fixation period.

Seston nitrogen and phosphorus decreased to minima 1-1.5 months after the decrease in dissolved inorganic nitrogen in 1974. In 1976 this occurred before and simultaneously with the decrease in inorganic nitrogen. Seston nitrogen minima (36 and 38 $\mu\text{g N mg DW}^{-1}$, and 0.6 and 0.6 mg N l^{-1}) appeared immediately before the commencement of acetylene reduction in 1974 and 1976 (Fig. 6). During 1978, low seston nitrogen per unit dry weight (41 $\mu\text{g N mg DW}^{-1}$) was found in the middle of August. Seston phosphorus per unit dry weight then reached the lowest values over all study years. At the same time non-heterocystous Aphanothece clathrata W. et G.S. West quadrupled its fresh weight, and seston N and P per liter reached maxima.

Concentrations of seston nitrogen, and seston phosphorus per liter increased during N_2 -fixation periods. For seston phosphorus per unit dry weight this only occurred in 1974 (Fig. 6). The seston N:P ratio decreased to 7:1 and 12:1 at the start of N_2 fixation in 1974 and 1976, but then increased to 15-21:1 during periods of intense N_2 fixation in 1974, 1976 and 1978. Total N:P ratios in the lake water usually amounted to $\geq 20:1$ during vegetation periods, but decreased to 17:1 before the start of nitrogen fixation in 1973, 1974 and 1978.

Total phytoplankton fresh weight varied between 1.6 and 32 mg l^{-1} during May-Oct in the four study years (Fig. 6). Highest densities were found in connection with N_2 fixation, and usually to a high proportion (maximally 80, 75, 95, and 10% in 1973, 1974, 1976, and 1978) consisted of heterocystous algae. The proportion of phytoplankton in total seston was 3-11% (estimated on dry weight basis; dry weight of diatoms, and other algae, was calculated to 30% and 15%, respectively, of fresh weight according to Winberg et al. 1971). No statistically significant correlation ($p > 0.05$) was found between phytoplankton and seston dry weight.

Heterocystous blue-green algae mainly occurred during nitrogen-fixation periods. Four species were represented, Aphanizomenon flos-aquae, Anabaena lemmermannii, A. viguieri, and A. spiroides var. spiroides, but no general successional pattern was found between them (Fig. 7). In 1976 A. gracile was the only heterocystous alga present. A. gracile also occurred during autumn through spring in 1974/75 and 1975/76, although then very few heterocysts were found.

Multiple linear regression analysis (with manual backward selection) of diurnal acetylene reduction per unit lake area over the whole years on 15 environmental and 5 phytoplankton variables showed that in individual years there were highly significant correlation between activity and the dominating heterocystous blue-green alga(e) (Tab. 2A). In 1974, however, the highest F value was assigned to total phosphorus. Over the four study years pH and total biomass of heterocystous algae were the only variables significantly correlated to daily acetylene reduction. No correlation was found between acetylene reduction and biomass of Aphanothece clathrata ($r^2 = 0.2$, $p > 0.1$).

When restricted to acetylene-reduction periods, multiple regression of maximum specific nitrogenase activity in the water column ($\text{mol C}_2\text{H}_4 \text{ mg FW}^{-1} \text{ h}^{-1}$) was significantly correlated to fresh weight of A. gracile over the four investigation seasons, to temperature in 1973, to A. spiroides in 1974, and to nitrate in 1978. In 1976 no significant relations were found (Tab. 2B). In 1973 and 1976, maximum development of heterocystous algae and acetylene reduction coincided with periods of warm, dry weather preceded by rain. However, acetylene reduction could not be shown to be correlated to light intensity, and precipitation during 10 days before sampling.

Between-years variation, and effects of lake management

During the post-restoration period 1972-1978 mean summer concentrations of total phosphorus and nitrogen decreased with about 25% (Tab. 3). Mean seston phosphorus was reduced with c. 40% between 1974/76 and 1978, while seston nitrogen was fairly stable. This resulted in an increase in the seston N:P ratio from 11-12:1 to 18:1. Although mean summer concentrations of dissolved inorganic nitrogen (DIN) decreased between 1969 and 1972, the annual cycles were characterized by high autumn-winter-spring and low summer concentrations both before and after restoration (Fig. 6 and 8). Mean freshweight of heterocystous blue-green algae varied between 2.6 and 5.8 mg l^{-1} during summers 1972-1976, and decreased to 1.2 and 0.3 mg l^{-1} during 1977 and 1978, respecti-

vely. No such decrease was registered for the total phytoplankton biomass, since the decline in heterocystous blue-greens was balanced by increased density of Aphanothece clathrata and other nanoplankton.

After the restoration acetylene reduction showed a declining trend (Fig. 6, Tab. 4). Estimated annual activity per unit lake area during 1974 and 1976 was about half of that in 1973, and decreased to a hardly detectable level in 1978. Mean nitrogenase activity per unit freshweight and day during nitrogen-fixation periods, was much higher in 1973 than during other years. Remarkably, the specific nitrogenase activity remained at the same level in 1978 as in 1974 and 1976.

No nitrogen-fixation studies were performed during the pre-restoration period. On basis of presence of heterocyst-carrying algae, concentrations of nitrate, ammonia and phosphate, and temperature, the potential nitrogen-fixation periods were determined (Fig. 8). Nitrogenase activity was then estimated according to two alternatives. Specific nitrogenase activities found during rise, maximum and decline of acetylene reduction in Lake Trummen 1973 and 1974 ($0.2\text{--}5 \text{ mmol C}_2\text{H}_4 \text{ g FW}^{-1} 24 \text{ h}^{-1}$) were applicated to fresh weight of heterocystous algae to give acetylene reduction per unit lake area per day. When oxygen saturation was 110–150% and >150% calculated rates were reduced with 60% and 70%, respectively (Shindler et al. 1980). The alternative method involved the use of specific nitrogenase rates from the nearby Lake Södra Bergundasjön, demonstrating characteristics similar to those of Lake Trummen before restoration. In this lake specific rates ($0.1\text{--}0.5 \text{ mmol C}_2\text{H}_4 \text{ g FW}^{-1} 24 \text{ h}^{-1}$) did not vary as much as in Lake Trummen (Leonardson & Bengtsson 1978). No correction was made for oxygen supersaturation. Resulting estimates per unit lake area per year ranged from values comparable to those of 1973, to values one order of magnitude higher (Tab. 4). Rates calculated on basis of activities in Lake Södra Bergundasjön were much less than those based on 1973 and 1974 Lake Trummen data.

Annual contributions by heterocystous blue-green algae to the nitrogen input of Lake Trummen were estimated to 2200, 1100, 1250 and 75 kg N, corresponding to 3.4, 1.7, 1.9 and 0.12 g N m^{-2} during 1973–1978 (Tab. 4). The annual amounts of N_2 fixed in the pelagial zone (cf. Digerfeldt 1972) before restoration were roughly estimated to 2000–33000 and 2000–12000 kg N, or 3.8–59 and $3.4\text{--}21 \text{ g N m}^{-2}$, during 1968 and 1969, respectively.

During the post-restoration period predominating species of heterocystous algae shifted (Fig. 7). After 1974 Anabaena lemmermannii was very sparse, and A. viguieri and Aphanizomenon gracile made up more important parts of the phytoplankton assemblage for some years. Anabaena spiroides var. spiroides only occurred occasionally. During 1975 and 1978 small non-heterocystous algae made up 69 and 47%, respectively, of the total phytoplankton biomass (Cronberg 1982).

During 1976-1978 populations of roach (Rutilus rutilus L.) and bream (Abramis brama L.) were reduced by use of rotenone and large fyke nets. Pike and perch caught were returned to the lake. Totally, 13400 kg fish was removed. From late spring 1977 throughout summer 1978, 8500 kg (c. 100 kg ha⁻¹) was caught, of which 70% was bream. During spring 1977 fish immigrated from downstream Lake Väckjösjön, partly neutralizing the fish-elimination effects. Also, in 1975 a large number of fish was observed immigrating into the lake (G. Andersson, pers. comm.).

DISCUSSION

Vertical variation

Nitrogen fixation (acetylene reduction) is a physiologically expensive process. Its day-time energy demand is mainly covered by photophosphorylation (Bottomley & Stewart 1977, Postgate 1978), while its requirement for reductant may be supported by both light-dependent and dark processes (Yates & Eady 1980). Since light intensity in lakes decreases at increasing water depth, photosynthesis and ATP supply to nitrogen fixation decrease, which results in lowered specific nitrogenase activity at greater depths (Dugdale & Dugdale 1962). On most sampling dates in Lake Trummen both specific acetylene reduction (Fig. 1C) and phytoplankton primary production (Gelin & Rippl 1978) agreed with this pattern. Due to supply of stored energy, reductant and carbon to nitrogenase even at low light intensity and in the aphotic zone (cf. Fay 1976), the vertical decrease of acetylene reduction usually was smaller than that of primary production. In addition, variations in light, carbon uptake and turbulence during hours preceding incubation on different sampling dates probably resulted in varying activities in incubation vessels located at greater water depths (cf. Dugdale & Dugdale 1962), and caused that no significant correlation ($p > 0.05$) was found between the vertical development of specific acetylene reduction and decrease in light intensity. However, correlations between seasonal fluctuations in lake-water pH (dependent on phytoplankton

photosynthesis) and acetylene-reduction rates (Tab. 2A), and between diurnal acetylene reduction and irradiance on the lake surface (Fig. 4) suggest couplings between light, photosynthesis and acetylene reduction. The correlation between vertical gradients of specific activity and phosphate was significant, but phosphate concentrations only may explain a minor proportion of the vertical variations in specific acetylene reduction.

Gelin & Riopl (1978) found that chlorophyll concentrations at 0.2 m did not differ significantly from concentrations in mixed samples from 0-1.5(2) m water depth in Lake Trummen, which agrees with results presented in this paper. Walsby (1972) concluded that most planktonic blue-green algae possess gas vacuoles by which they may regulate their buoyancy, and Reynolds (1967, cited by Fogg et al. 1973) found that algal species forming larger colonies have higher flotation potential than those which are smaller or form small colonies. In Lake Trummen Anabaena species, either forming large tangled balls of filaments (A. lemmermannii, A. spiroides), or straight filaments of large diameter (6-7 μm , A. viguieri), rose to the lake surface on dates when Aphanizomenon gracile, having small-diameter (2-3.5 μm) single filaments, was evenly distributed throughout the water profile. Although present in the algal assemblage during all study years, A. gracile only was found to form surface accumulations on three occasions with extremely calm weather.

Horne & Wrigley (1975) found that surface accumulation of algae was a prerequisite for wind-induced heterogeneous horizontal distribution in phytoplankton. A consequence of the almost total lack of surface accumulations of phytoplankton in Lake Trummen is that patchiness should have occurred only rarely. Samples taken centrally in the lake would then be representative for the whole lake area. This agrees with the conclusion of Cronberg (1982), derived from Melosira studies.

Similar vertical patterns of specific acetylene reduction as in Lake Trummen have been described for several lakes and marine areas, e.g. Lake Erken (L  nnergren et al. 1974), Lake Mendota (Peterson et al. 1977, Vanderhoef et al. 1975, Torrey & Lee 1976), Clear Lake (Horne 1979), the North Pacific Ocean (Mague et al. 1977), the Sargasso and Caribbean Seas (Carpenter & Price 1977) and the Baltic Sea Archipelagoes (Brattberg 1977, Lindahl et al. 1980).

Surface inhibition of specific nitrogenase activity around noon has been assumed to be the result either of oxygen inhibition, which may occur even at pO_2 below 0.2 (Stewart & Pearson 1970), photooxidative inactivation or dest-

ruption of pigments (Golterman 1975, Horne 1979), or photorespiratory competition with nitrogenase for reducing power (Lex et al. 1972). In the majority of the surface inhibition cases in Lake Trummen, oxygen saturation at 0.2 m varied between 95 and 105%, the sky was more or less cloudy, and pH c. 8. Under these conditions oxygen inhibition is the most probable process to have hampered nitrogenase activity. Only on a few sampling dates, primarily 16, 28, and 29 Aug 1973, the conditions for photorespiration seem to have been fulfilled: (1) oxygen reached saturation values of 120-145% at noon, (2) pH values of 9.4-9.5 displaced the $\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$ equilibrium towards CO_3^{2-} , resulting in much lowered availability of carbon dioxide to algae (Total $\text{CO}_2 = 220 \mu\text{mol l}^{-1}$, free $\text{CO}_2 = 0.17 \mu\text{mol l}^{-1}$), and (3) high light intensities occurred in the surface water. However, even on these days one cannot exclude the possibility of oxygen inhibition or photooxidation as being the crucial processes. It must also be kept in mind that the artificial environment created in the experimental vessels, including the inability of algae to circulate in the water column, might result in conditions more extreme than in the lake even during short exposures (cf. Ganf & Horne 1975, Marra 1978).

Diurnal variation

The day-time displacement of carbon fixation and acetylene reduction in Lake Trummen shows similarities to results found in other *Anabaena*-dominated lakes (Ashton 1979, Paerl 1979, Paerl & Kellar 1979), and in laboratory cultures with *Anabaena flos-aquae* and *A. oscillarioides* (Paerl & Kellar 1979). The pattern was suggested to be due to higher oxygen sensitivity of carbon-uptake processes than of nitrogen fixation (Paerl & Kellar 1979). Thus, carbon fixation was assumed to be more inhibited by oxygen during afternoon when supersaturation was higher than before noon, while the opposite was suggested to be true for nitrogen fixation. By the resulting partial separation of the two processes over the day *Anabaena* reduces competition for light-generated reductant between carbon and nitrogen fixation, and optimizes C and N uptake (Paerl 1979). Paerl's results agree with those of Stewart & Pearson (1970). Both reported that initial oxygen inhibition of nitrogenase was reversible within a few hours. Paerl (1979, Paerl & Kellar 1979) also found that carbon uptake remained depressed for longer time than nitrogen fixation in *Anabaena* that had been exposed to supersaturated levels of oxygen. Moreover, bacteria found around heterocysts might have subdued oxygen inhibition, and enhanced nitrogenase activity after inhibition (Paerl & Kellar 1978). Paerl (1979) also suggested it to be advantageous for algae to fix carbon before nitrogen, since in order not to inhibit nitrogen fixation the formed ammonia has to be coupled

to carbon skeletons produced by photosynthesis (cf. Fogg et al. 1973, Wolk 1968).

Several authors found higher rates of acetylene reduction in the morning than in the afternoon, and suggested that the decreased afternoon activity was caused by oxygen inhibition, photooxidation of cells or pigments, or photorespiration (Vanderhoef et al. 1975, Torrey and Lee 1976, Ganf and Horne 1975, Horne 1975). Also evidence for two and three diurnal maxima were shown by Vanderhoef et al. (1975), Horne (1975), Mague et al. (1977) and Peterson et al. (1977). Midday depression of nitrogenase activity was also shown to be at least partly affected by the sinking of blue-green algae (Ganf & Horne 1975, Vanderhoef et al. 1975, Peterson et al. 1977). This resulted in lower acetylene reduction per unit water volume at the lake surface, but did not always lead to lower specific activity at the greater water depth, since lower light intensity may result in lower light inhibition.

Nocturnal nitrogen fixation (^{15}N method and acetylene reduction) was described from several lakes and marine areas. Estimated rates usually varied between 10 and 30% of the total diurnal activity (e.g. Horne 1975, Paerl 1979), although a few measurements indicated much higher activity (Mague et al. 1977, Horne 1975). Laboratory studies have shown the dependence of nocturnal acetylene reduction on previous light conditions, and carbon assimilation during the preceding light period (Fay 1976).

Seasonal variation of acetylene reduction

Correlation between fresh weight of Aphanizomenon and Anabaena, and acetylene reduction agreed with the hypothesis that nitrogen fixation in freshwaters mainly depends on algae carrying heterocysts (Stewart et al. 1967, Fay et al. 1968, Wolk et al. 1974). Since heterocysts lack the O_2 -evolving photosystem II, nitrogenase produced in heterocysts is to high extent protected from exposure to inhibiting concentrations of oxygen (Fogg et al. 1973). However, Singh (1973) found that a non-heterocystous alga, Aphanothece sp., fixed dinitrogen under aerobic conditions. This anomaly, occurring in a small number of chroococcal blue-green algal species, has been suggested to be due to at least three mechanisms protecting nitrogenase from oxygen: compartmental separation of photosystem II and N_2 fixation within vegetative cells, temporal separation of photosynthesis and nitrogen fixation, and protective respiration (Postgate 1978, Gallon 1981). Although Aphanothece clathrata appeared in Lake Trummen, and in 1978 was the predominating alga, it did not fix

N_2 . This was probably not caused by oxygen inhibition, since on occasions in 1976 and 1978 when A. clathrata occurred pH values around 8 indicated that oxygen saturation would have been close to 100%. However, many small-sized algae have a more efficient nutrient uptake at low ambient nutrient levels (cf. Gelin & Rip1 1978, Shindler et al. 1980, Schlesinger et al. 1981), and lower subsistence quotas (q_0 ; Shuter 1978), than large-sized algae. Thus, it is possible that prevailing concentrations of nitrate and ammonium were sufficiently high to repress nitrogenase activity in A. clathrata.

Laboratory (e.g. Fogg 1949, Murry & Benemann 1979) and in-situ (e.g. Horne & Fogg 1970, Schindler & Fee 1974, Horne et al. 1979) studies demonstrated the general relation between low concentration of dissolved inorganic nitrogen in the environment, and heterocyst production and N_2 fixation in blue-green algae. Although dissolved inorganic nitrogen in Lake Trummen decreased in spring and increased in autumn, no significant relation to acetylene reduction was found by means of regression analysis. This was due to (1) that the concentration of dissolved inorganic nitrogen was low and relatively stable during summer, while acetylene-reduction rates varied several magnitudes, and (2) complex interactions between dissolved inorganic nitrogen and seston nitrogen at the start and end of N_2 -fixation periods.

Seston-nitrogen data may be interpreted along two lines, both leading to the same conclusion. Early-summer minima of seston nitrogen per unit dry weight agree with nitrogen content of blue-green and other algae during nitrogen-deficient conditions (Gerloff & Skoog 1957, Healey 1975, Ahlgren 1977). Staub (1961) considered 5-6% nitrogen (on dry weight basis) in Oscillatoria rubescens DC. to be the critical level below which no growth is possible (q_0). Accordingly, minima could be interpreted as indicating nitrogen deficiency in the phytoplankton of Lake Trummen, and initiating the development of nitrogen-fixing algae.

Evidence for high dependence of phytoplankton productivity on nutrients generated by the turnover of seston was found in oligotrophic and eutrophic lakes (Dugdale & Goering 1967, Bloesch et al. 1977, Axler et al. 1981). Otsuki & Hanya (1972a, 1972b) found that after the initial rapid mineralization of dead algae, the proportion of refractory particulate nitrogen was decomposed very slowly. In Lake Trummen both the total quantity of seston nitrogen in the water, and the nitrogen contents of particles reached minima simultaneously. Then the flow of nitrogen made available for phytoplankton uptake by decomposition of seston would have been minimized both because of the decreasing

amount of seston available for mineralization, and because of the decreasing proportion of labile seston N. Obviously, the start of dinitrogen fixation was not only regulated by the declining concentration of dissolved inorganic nitrogen, but even turnover of the seston-nitrogen pool was an important process moderating the nitrogen availability to phytoplankton. In 1978, other factors than nitrogen, e.g. competition with small-sized blue greens, or lower P availability, may have restricted the development of heterocystous algae.

An analogous conclusion was derived from data from the nearby Lake Södra Bergundasjön, where in 1975 seston nitrogen successively decreased from 1.7 to 0.7 mg N l⁻¹ during two months after the mean concentration of dissolved inorganic nitrogen reached 30 µg N l⁻¹. The minimum seston-N concentration was found in connection with the commencement of N₂ fixation (Leonardson unpubl., cf. Leonardson & Bengtsson 1978).

Phosphate and seston-phosphorus concentrations indicated low phosphorus availability and phosphorus deficiency (cf. Healey 1975) after the spring phytoplankton maxima, and before nitrogen fixation started. However, according to N:P ratios (DIN:PO₄-P <5:1, seston N:P 7:1 in 1974 and 12:1 in 1976) nitrogen was the limiting nutrient during this time (cf. Claesson 1978), representing the development of non-nitrogen-fixing algae. Simultaneously with the start of nitrogen fixation in 1974, concentrations of seston nitrogen per liter and per unit dry weight, and seston phosphorus per liter and per unit dry weight, increased (Fig. 6). Thus, while nitrogen deficiency was overcome by means of biological N₂ fixation, increased phosphorus availability, reflected in increased seston phosphorus in the lake water, seemed to be a prerequisite for the rapid growth and intense dinitrogen fixation in heterocystous algae during this year (cf. Schindler 1977). However, during spring 1976 seston phosphorus per liter and per unit dry weight was lower than in 1974, and did not increase until after the nitrogen-fixation maximum in June. Thus, the results from 1976 suggests that phosphorus did not regulate the start of N₂ fixation in either year.

In 1973 the late-summer decline of acetylene reduction coincided with increased concentration of dissolved inorganic nitrogen and decreasing temperature in the lake water. In 1974 and 1976 seston nitrogen per unit dry weight increased during N₂ fixation, and indicated high nitrogen status of algae. This would have allowed N₂ fixers to multiply. That this did not happen suggests that other factors than nitrogen limited their development. Besides DIN:PO₄-P ratios (<=5) indicated nitrogen deficiency for further algal

growth during Aug and Sept in 1974 and 1976 (cf. Claesson 1978), why acetylene reduction should have continued. However, seston N:P ratios indicated at least weak phosphorus deficiency in phyto-plankton (cf. Shindler et al. 1980) during the N_2 -fixation periods in 1974, 1976 and 1978. In 1974 the decline in acetylene reduction was simultaneous to the heavy drop in seston phosphorus both per liter and per unit dry weight (Fig. 6), supporting the phosphorus-deficiency hypothesis for this year. Moreover, minima in total Fe:seston dry weight and total Fe:phytoplankton biomass coincided with acetylene-reduction maxima in 1973 and 1974, and with the second acetylene-reduction peak in 1976. The declines in nitrogenase activity could thus have been caused by either phosphorus or iron limitation. Wurtsbaugh & Horne (1983) found evidence for iron-limited acetylene reduction in a natural population of Aphanizomenon flos-aquae. However, Murphy et al. (1976) suggested that some blue-green algae, e.g. Anabaena, can overcome such deficiencies by producing iron-selective chelators. To experimentally determine the influence of phosphorus and iron on the duration and rate of acetylene reduction, several in-situ enrichment experiments were started during summer 1976. Due to hard weather and bird interactions the experiments were repeatedly disturbed, and had to be interrupted without producing any results.

Between-years variation, and effects of lake management

The restoration of Lake Trummen resulted in great changes in water chemistry, and phytoplankton assemblage structure and biomass (Tab. 3; Cronberg 1982). Effects were most conspicuous during the first post-restoration year, 1972, after which water quality improved at a slower rate. Concentrations of ions critical for the development of phytoplankton, and for the regulation of nitrogen fixation, e.g. ammonium, nitrate, phosphate and iron (Fig. 6 and 8, Tab. 3), were reduced as a result of decreased internal release from the oligotrophic deeper sediment layers exposed after restoration (Bengtsson & Fleischer 1971, Bengtsson et al. 1975).

Conditions for nitrogen fixation were favourable during the pre-restoration period, and similar to those in Lake Södra Bergundasjön after sewage diversion (Leonardson & Bengtsson 1978). While lake-water concentrations of nitrate and ammonium were low during summer, concentrations of phosphorus and iron were high (Fig. 8). Due to low oxygen concentrations at the sediment surface, or because of high pH (9.6-10.4) induced by photosynthesis (Boström et al. 1982), phosphate even increased during phytoplankton bloom periods. Estimated rates of nitrogen fixation for 1968 and 1969 indicate that the amounts of nitrogen

supplied to the lake could have been much higher than during the post-restoration period. It is probable that acetylene reduction was higher than the lower range values, which were estimated from specific activities in Lake Södra Bergundasjön. This assumption was based on the fact that in this lake nitrogen fixation seldom covered the demands of heterocystous algae, and that this probably was caused by elevated ammonium and nitrate levels. Moreover, during the N_2 -fixation periods 1968 and 1969 in Lake Trummen, total lake-water nitrogen increased with c. 4 mg l^{-1} (corresponding to 4.4 g m^{-2}), which was much more than occurred during the postrestoration period. However, the coincidence of the nitrogen increase with release of phosphorus from the sediment suggests the origin to be at least two sources. Finally, although A. spiroides var. crassa dominated in Lake Trummen 1968 and in Lake Södra Bergundasjön 1975 and 1976, it is possible that different clones as well as different species of algae have different nutrient-uptake characteristics. This makes an evaluation of the nitrogen fixation in Lake Trummen before restoration even more difficult.

After restoration, low phosphate levels in Lake Trummen water suggest high phosphorus exploitation by algae. Stewart & Alexander (1971) and Lundgren (1978) found that phosphorus additions to cultures of blue-green algae, and to natural periphyton assemblages stimulated acetylene reduction. Several other authors suggested phosphorus to regulate planktonic nitrogen fixation (Vanderhoef et al. 1974, Leonardson & Bengtsson 1978). Healey (1975) considered $5 \text{ ug P mg DW}^{-1}$ to indicate extreme phosphorus deficiency in algae. In Lake Trummen seston phosphorus usually was below this limit during 1974, 1976 and 1978 (Fig. 6, Tab. 3). Analogous to the seston-nitrogen discussion it may be argued that the long-term decrease in total seston P per liter and per unit seston dry weight resulted in lower amounts of P released via seston decomposition. Phosphorus deficiency in algae, therefore, increased during the post-restoration period in Lake Trummen, and not only influenced the seasonal nitrogen-fixation pattern, but also long-term fixation rates.

Although total iron decreased much between 1968/69 and 1972/1978, Fe:phytoplankton fresh weight ratios were 2-3 times higher after restoration. This suggests that iron only exceptionally may have limited phytoplankton growth and nitrogen fixation.

The phytoplankton assemblage structure changed between pre- and post-restoration periods (Cronberg 1982). Among blue-green algae small-sized species, e.g. Aphanothece clathrata, Cyanodictyon imperfectum, Aphanizomenon gracile,

Anabaena lemmermannii, A. spiroides var. spiroides, A. viguieri, replaced large-sized species, e.g. Microcystis spp., Aphanizomenon flos-aquae (L.) Ralfs, Anabaena solitaria f. smithii Kom., A. spiroides var. crassa Lemm., Oscillatoria limnetica var. acicularis Nygaard. Also biomass of blue-green and green algae declined, and heavy summer blooms causing surface scums of blue greens ceased (Tab. 3). Changes also occurred in most other algal phyla.

Enclosure experiments in Lake Trummen (by Dr. G. Andersson) revealed important relations between species and biomass of fish, and lake trophic status (Andersson et al. 1978, Cronberg 1982). Introduction of bream and roach was followed by increased phosphorus and nitrogen release from the sediment, increased number of small zooplankton, and increased biomass of blue-green algae. The exclusion of roach and bream, or introduction of perch, resulted in large zooplankton species, low biomass of phytoplankton, and low nutrient concentrations.

The almost total lack of heterocystous algae and nitrogen fixation during summer 1978 most probably was due to the large-scale fish elimination started in 1976 (Andersson et al. 1978, Cronberg 1982). It was not attributable to meteorological factors, since no similar development was found in adjacent Lake Södra Bergundasjön. Besides, during 1979 through 1981 fishing efforts were reduced, and Anabaena and Aphanizomenon reappeared at high densities (Cronberg pers. comm.). The proportion of fish removed during May 1977 through summer 1978 probably corresponded to 10-20% of the total bream and roach biomass (based on roach biomass determinations in a South Swedish eutrophic lake, Persson MS). The replacement of Bosmina longirostris by low numbers of the larger, predator-sensitive B. coregoni during 1978 was taken as evidence for an ecosystem response in oligotrophicating direction (Andersson 1979, cf. Brooks & Dodson 1965). It is suggested that the presence of Aphanothece clathrata from the time of diatom maximum in medio June was enhanced by reduced predation pressure by fish on predatory plankton crustacea, e.g. increased numbers of cyclopoid copepods and Leptodora kindtii was found this year compared to 1976 (G. Andersson pers. comm.). This probably resulted in increased predation on small filtering zooplankton, why grazing on small-sized A. clathrata was reduced. If small-sized algae take up nutrients more efficiently than large-sized ones (see above), this would explain why A. clathrata, once established, was predominating in the phytoplankton assemblage during all summer 1978. In addition, slower phosphorus release from the sediment due to reduced number of bream, would have strengthened the competitive

advantage of the alga. As shown in Fig. 6, small increases in phosphate, e.g. in July and Aug, resulted in high biomass increases in A. clathrata. This probably was due to low phosphorus demand per cell, as indicated by the very low seston P per unit dry weight. Consequently, heterocystous blue-green algae were outcompeted for phosphorus, and nitrogen fixation efficiently inhibited by phosphorus deficiency (cf. Petersen Holm et al. 1983).

Annual amounts of fixed dinitrogen during the post-restoration period in Lake Trummen ($3.4\text{--}0.12\text{ g N m}^{-2}$) was within ranges found by most other authors (e.g. Horne & Fogg 1970, Granhall & Lundgren 1971, Horne & Goldman 1972, Lännergren et al. 1974, Torrey & Lee 1976, Leonardson & Bengtsson 1978, Flett et al. 1980), while the upper ranges estimated for 1968 and 1969 were significantly higher. In Lake George, Uganda, Horne & Viner (1971) found $4.4\text{ g N m}^{-2}\text{ year}^{-1}$, which is the highest rate hitherto reported. Annual variation may be great within the same lake, e.g. from Lake Erken 3.0 and 0.33 g N m^{-2} were reported (Granhall & Lundgren 1971, Lännergren et al. 1974). A preliminary estimate of the annual nitrogen input to Lake Trummen through inlets amounted to c. 7000 kg N for 1974 (Ripl unpubl.). Assuming the 1974 input value to be valid for the post-restoration years, gives estimates of the percentage contribution by N_2 fixation to the total nitrogen input (incl. N_2 fixation) of 24, 14, 15 and 1% for 1973, 1974, 1976 and 1978. Similar estimates ranged between c. 1% contributed to Lake Windermere (Horne & Fogg 1970), and 60–70% in Lake Erken (Granhall & Lundgren 1971). In Lake George, the annual contribution was 33%. Although dissolved inorganic nitrogen concentrations were low in this equatorial lake, nitrogen generated by turnover of the high seston pool (Greenwood 1976) may have repressed nitrogen fixation. The high percentages during 1973–1976 in Lake Trummen confirm the importance of the N_2 -fixation process as a biological nutrient regulator (cf. Schindler 1977). This is even more conspicuous if considered that nitrogen fixation – like in most lakes in the temperate vegetation zone – only occurred during summer, when the input of nitrogen from other external sources was low (cf. Leonardson & Bengtsson 1978).

It is concluded that management measures induced great changes in Lake Trummen ecosystem. Before the restoration conditions for dinitrogen fixation were favourable, i.e. low concentrations of ammonium and nitrate, and high levels of phosphate and iron during periods with heterocystous blue-green algae. After restoration N_2 fixation successively decreased because of increasing phosphorus deficiency, or occasional late-summer iron depletion. Due to intensive fish elimination during 1977–1978, small-sized Aphanothece clathrata

predominated and probably competed severely for phosphorus. The development of N_2 fixers was then depressed, and nitrogen fixation the lowest for the period.

ACKNOWLEDGMENTS

I thank Prof. S. Björk, Dr. G. Andersson, and Mr. G. Gahnström for valuable comments on the manuscript. Mr. J. Jonasson helped with field work and GC analyses, and Miss A. Fritzon and Mr. J. Bertilsson did the plankton analyses. Miss K. Nilsson analyzed seston phosphorus, and Dr. T. Persson helped with multiple regression analyses. Prof. W. Ripl, and Drs. G. Andersson and G. Cronberg kindly supplied unpublished data. I am also grateful to Drs. G. and I. Ahlgren allowing me access to the Carlo Erba Elemental Analyzer at the Institute of Limnology in Uppsala. Financial support was given by The National Swedish Environment Protection Board and The Royal Swedish Academy of Science.

REFERENCES

- AHLGREN, G. (1976): Comparison between methods for determination of total phosphorus in lake water. (In Swedish, English summary.) - Vatten 32:38-44.
- AHLGREN, G. (1977): Growth of Oscillatoria agardhii in chemostat culture. 1. Nitrogen and phosphorus requirements. - Oikos 29:209-224.
- ANDERSSON, G. (1979): Effects of fish on trophic conditions in eutrophic lakes. Final report. - Inst. of Limnology, Lund. Mimeographed. 22 p. (In Swedish, English summary.)
- ANDERSSON, G., BERGGREN, H., CRONBERG, C. & GELIN, C. (1978): Effects of planktivorous and benthivorous fish on organisms and water chemistry in eutrophic lakes. - Hydrobiologia 59:9-15.
- ANDERSSON, G., BERGGREN, H. & HAMRIN, S. (1975): Lake Trummen restoration project. III. Zooplankton, macrobenthos and fish. - Verh. Internat. Verein. Limnol. 19:1097-1106.
- ASHTON, P.J. (1979): Nitrogen fixation in a nitrogen-limited impoundment. - J. Wat. Poll. Cont. Fed. 51:570-579.
- AXLER, R.P., REDFIELD, G.W. & GOLDMAN, C.R. (1981): The importance of regenerated nitrogen to phytoplankton productivity in a subalpine lake. - Ecology 62:345-354.
- BENGTTSSON, L. & FLEISCHER, S. (1971): Sediment investigations in the lakes Trummen and Hinnasjön 1968-1970. (In Swedish, English summary.) - Vatten 27:73-94.

- BENGSSON, L., FLEISCHER, S., LINDMARK, G. & RIPL, W. (1975): Lake Trummen restoration project. I. Water and sediment chemistry. - Verh. Internat. Verein. Limnol. 19:1080-1087.
- BJÖRK, S. (1972): Swedish lake restoration program gets results. - Ambio 1:153-165.
- BJÖRK, S. ET AL. (1972): Ecosystem studies in connection with the restoration of lakes. - Verh. Internat. Verein. Limnol. 18:379-387.
- BLOESCH, J., STADELMANN, P. & BÜHRER, H. (1977): Primary production, mineralization, and sedimentation in the euphotic zone of two Swiss lakes. - Limnol. Oceanogr. 22:511-526.
- BOSTRÖM, B., JANSSON, M. & FORSBERG, C. (1982): Phosphorus release from lake sediments. - Arch. Hydrobiol. Beih. Ergebn. Limnol. 18:5-59.
- BOTTOMLEY, P.J. & STEWART, W.D.P. (1977): ATP and nitrogenase activity in nitrogen-fixing heterocystous blue-green algae. - New. Phytol. 79:625-638.
- BRATTBERG, G. (1977): Nitrogen fixation in a polluted brackish water archipelago. - Ambio Special Report 5:27-42.
- BROOKS, J.L. & DODSON, S.I. (1965): Predation, body size, and composition of plankton. - Science 150:28-35.
- CARPENTER, E.J. & PRICE IV, C.C. (1977): Nitrogen fixation, distribution, and production of Oscillatoria (Trichodesmium) spp. in the western Sargasso and Caribbean Seas. - Limnol. Oceanogr. 22:60-72.
- CLAESSON, A. (1978): Research on recovery of polluted lakes. Algal growth potential and the availability of limiting nutrients. - Ph. D. thesis. University of Uppsala, Sweden. ISBN 91-554-0754-4.
- CRONBERG, G. (1982): Phytoplankton changes in Lake Trummen induced by restoration. Long-term whole-lake studies and food-web experiments. - Folia Limnologica Scandinavica 18. 119 pp.
- CRONBERG, G., GELIN, C. & LARSSON, K. (1975): Lake Trummen restoration project. II. Bacteria, phytoplankton and phytoplankton productivity. - Verh. Internat. Verein. Limnol. 19:1088-1096.
- DIGERFELDT, G. (1972): The post-glacial development of Lake Trummen. Regional vegetation history, water level changes and palaeolimnology. - Folia Limnologica Scandinavica 16. 104 pp.
- DUGDALE, V.A. & DUGDALE, R.C. (1962): Nitrogen metabolism in lakes. II. Role of nitrogen fixation in Sanctuary Lake, Pennsylvania. - Limnol. Oceanogr. 7:170-177.
- DUGDALE, R.C. & GOERING, J.J. (1967): Uptake of new and regenerated forms of nitrogen in primary productivity. - Limnol. Oceanogr. 12:196-207.
- FAY, P. (1976): Factors influencing dark nitrogen fixation in a blue-green alga. - Appl. Environ. Microbiol. 31:376-379.

- FAY, P., STEWART, W.D.P., WALSBY, A.E. & FOGG, G.E. (1968): Is the heterocyst the site of nitrogen fixation in blue-green algae? - *Nature* 220:810-812.
- FLETT, R.J., HAMILTON, R.D. & CAMPBELL, N.E.R. (1976): Aquatic acetylene-reduction techniques: solutions to several problems. - *Can. J. Microbiol.* 22:43-51.
- FLETT, R.J., SCHINDLER, D.W., HAMILTON, R.D. & CAMPBELL, E.R. (1980): Nitrogen fixation in Canadian Precambrian Shield lakes. - *Can. J. Fish. Aquat. Sci.* 37:494-505.
- FOGG, G.E. (1949): Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon and nitrogen metabolism. - *Ann. Bot., N.S.* 13:241-259.
- FOGG, G.E., STEWART, W.D.P., FAY, P. & WALSBY, A.E. (1973): The blue-green algae. - Academic Press, London and New York. 459 pp.
- GALLON, J.R. (1981): The oxygen sensitivity of nitrogenase: a problem for biochemists and micro-organisms. - *Trends Biochem. Sci.* 61:19-23.
- GANF, G.G. & HORNE, A.J. (1975): Diurnal stratification, photosynthesis and nitrogen-fixation in a shallow, equatorial Lake (Lake George, Uganda). - *Freshwat. Biol.* 5:13-39.
- GELIN, C. (1975): Nutrients, biomass and primary productivity of nanoplankton in eutrophic Lake Vombsjön, Sweden. - *Oikos* 26:121-139.
- GELIN, C. & RIPL, W. (1978): Nutrient decrease and response of various phytoplankton size fractions following the restoration of Lake Trummen, Sweden. - *Arch. Hydrobiol.* 81:339-367.
- GERLOFF, G.C. & SKOOG, F. (1957): Nitrogen as a limiting factor for the growth of Microcystis aeruginosa in southern Wisconsin lakes. - *Ecology* 38:556-561.
- GOLTERMAN, H.L. (1975): Physiological limnology. An approach to the physiology of lake ecosystems. - Developments in water science. 2. Elsevier, Amsterdam.
- GRANHALL, U. & LUNDGREN, A. (1971): Nitrogen fixation in Lake Erken. - *Limnol. Oceanogr.* 16:711-719.
- GREENWOOD, P.H. (1976): Lake George, Uganda. - *Phil. Trans. R. Soc. Lond. B.* 274:375-391.
- HEALEY, F.P. (1975): Physiological indicators of nutrient deficiency in algae. - *Fish. Mar. Serv. Res. Dev. Tech. Rep.* 585:1-30.
- HORNE, A.J. (1975): Algal nitrogen fixation in Californian streams: diel cycles and nocturnal fixation. - *Freshwat. Biol.* 5:471-477.
- HORNE, A.J. (1979): Nitrogen fixation in Clear Lake, California. 4. Diel studies on Aphanizomenon and Anabaena blooms. - *Limnol. Oceanogr.* 24:329-341.

- HORNE, A.J. & FOGG, G.E. (1970): Nitrogen fixation in some English lakes.
- Proc. Roy. Soc. Lond. B. 175:351-366.
- HORNE, A.J. & GOLDMAN, C.R. (1972): Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. - Limnol. Oceanogr. 17:678-692.
- HORNE, A.J., SANDUSKY, J.C. & CARMIGGELT, C.J.W. (1979): Nitrogen fixation in Clear Lake, California. 3. Repetitive synoptic sampling of the spring Aphanizomenon blooms. - Limnol. Oceanogr. 24:316-328.
- HORNE, A.J. & VINER, A.B. (1971): Nitrogen fixation and its significance in tropical Lake George, Uganda. - Nature 232:417-418.
- HORNE, A.J. & WRIGLEY, R.C. (1975): The use of remote sensing to detect how wind influences planktonic blue-green algal distribution. - Verh. Internat. Verein. Limnol. 19:784-791.
- LEONARDSON, L. (1980): Interactions between Lugol's fixative and ethylene in the acetylene-reduction assay for nitrogenase activity in lake water.
- Can. J. Microbiol. 26:599-605.
- LEONARDSON, L. (1983): Effects of concentrating phytoplankton on the acetylene-reduction assay for nitrogenase activity. - Freshwat. Biol. 13:265-274.
- LEONARDSON, L. (In prep). Does N_2 fixation meet the nitrogen requirements of heterocystous blue-green algae in shallow eutrophic lakes? - Submitted to Oecologia (Berl.), 1984.
- LEONARDSON, L. & BENGTTSSON, L. (1978): Effects of sewage diversion in Lake Södra Bergundasjön. II. Phytoplankton changes and the role of nitrogen fixation. - Verh. Internat. Verein. Limnol. 20:2701-2707.
- LEX, M., SILVESTER, W.B. & STEWART, W.D.P. (1972): Photorespiration and nitrogenase activity in the blue-green alga, Anabaena cylindrica. - Proc. R. Soc. Lond. B. 180:87-102.
- LINDAHL, G., WALLSTRÖM, K. & BRATTBERG, G. (1980): Short-term variations in nitrogen fixation in a coastal area of the Northern Baltic. - Arch. Hydrobiol. 89:88-100.
- LUNDGREN, A. (1978): Nitrogen fixation induced by phosphorus fertilization of a subarctic lake. - Ecol. Bull. (Stockholm) 26:52-59.
- LÄNNERGREN, C., LUNDGREN, A. & GRANHALL, U. (1974): Acetylene reduction and primary production in Lake Erken. - Oikos 25:365-369.
- MAGUE, T.H., MAGUE, F.C. & HOLM-HANSEN, O. (1977): Physiology and chemical composition of nitrogen-fixing phytoplankton in the Central North Pacific Ocean. - Mar. Biol. 41:213-227.
- MARRA, J. (1978): Phytoplankton photosynthetic response to vertical movement in a mixed layer. - Mar. Biol. 46:203-208.

- MENZEL, D.W. & CORWIN, N. (1965): The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. - *Limnol. Oceanogr.* 10:280-282.
- MURRY, M.A. & BENEMANN, J.R. (1979): Nitrogenase regulation in Anabaena cylindrica. *Pl. Cell Physiol.*, Tokyo 20:1391-1401.
- MURPHY, T.P., LEAN, D.R.S. & NALEWAJKO, C. (1976): Blue-green algae: Their excretion of iron-selective chelators enables them to dominate other algae. - *Science* 192:900-902.
- OTSUKI, A. & HANYA, T. (1972a): Production of dissolved organic matter from dead green algal cells. I. Aerobic microbial decomposition. - *Limnol. Oceanogr.* 17:248-257.
- OTSUKI, A. & HANYA, T. (1972b): Production of dissolved organic matter from dead green algal cells. II. Anaerobic microbial decomposition. - *Limnol. Oceanogr.* 17:258-264.
- PAERL, H.W. (1979): Optimization of carbon dioxide and nitrogen fixation by the blue-green alga Anabaena in freshwater blooms. - *Oecologia (Berl.)* 38:275-290.
- PAERL, H.W. & KELLAR, P.E. (1978): Significance of bacterial-Anabaena (Cyanophyceae) associations with respect to N_2 fixation in freshwater. - *J. Phycol.* 14:254-260.
- PAERL, H.W. & KELLAR, P.E. (1979): Nitrogen-fixing Anabaena: Physiological adaptations instrumental in maintaining surface blooms. - *Science* 204:620-622.
- PERSSON, L. (MS): Effects of reduced interspecific competition on resource utilization of a perch (Perca fluviatilis) population. - Submitted to *Ecology*, 1984.
- PETERSON, R.B. & BURRIS, R.H. (1976): Conversion of acetylene reduction rates to nitrogen fixation rates in natural populations of blue-green algae. - *Anal. Biochem.* 73:404-410.
- PETERSON, R.B., FRIBERG, E.E. & BURRIS, R.H. (1977): Diurnal variation in N_2 fixation and photosynthesis by aquatic blue-green algae. - *Plant Physiol.* 59:74-80.
- PETERSON HOLM, N., GANF, G.G. & SHAPIRO, J. (1983): Feeding and assimilation rates of Daphnia pulex fed Aphanizomenon flos-aquae. - *Limnol. Oceanogr.* 28:677-687.
- POSTGATE, J. (1978): Nitrogen fixation. - *Studies in biology* no. 92. Edward Arnold, London. 67 pp.
- REYNOLDS, C.S. (1967): The breaking of the Shropshire meres. - *Shropshire Conservation Trust Bull.* 10:9-14.

- SCHINDLER, D.W. (1977): Evolution of phosphorus limitation in lakes. - Science 195:260-262.
- SCHINDLER, D.W. & FEE, E.J. (1974): Experimental Lakes Area: Whole-lake experiments in eutrophication. - J. Fish. Res. Board Can. 31:937-953.
- SCHLESINGER, D.A., MOLOT, L.A. & SHUTER, B.J. (1981): Specific growth rates of freshwater algae in relation to cell size and light intensity. - Can. J. Fish. Aquat. Sci. 38:1052-1058.
- SHINDLER, D.B., PAERL, H.W., KELLAR, P.E. & LEAN, D.R.S. (1980): Environmental constraints on *Anabaena* N_2 and CO_2 -fixation: Effects of hyperoxia and phosphate depletion on blooms and chemostat cultures. - In: J. BARICA & L.R. MUR, Eds. Developments in hydrobiology. Vol. 2. Hypertrophic ecosystems. Junk, The Hague, Netherlands, pp. 221-229.
- SHUTER, B.J. (1978): Size dependence of phosphorus and nitrogen subsistence quotas in unicellular microorganisms. - Limnol. Oceanogr. 23:1248-1255.
- SINGH, P.K. (1973): Nitrogen fixation by the unicellular blue-green alga *Aphanothece*. - Arch. Mikrobiol. 92:59-62.
- SOLORZANO, L. & SHARP, J.H. (1980): Determination of total dissolved phosphorus and particulate phosphorus in natural waters. - Limnol. Oceanogr. 25:754-758.
- STAUB, R. (1961): Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge *Oscillatoria rubescens* DC. - Schweiz. Z. Hydrol. 23:82-198.
- STEWART, W.D.P. & ALEXANDER, G. (1971): Phosphorus availability and nitrogenase activity in aquatic blue-green algae. - Freshwat. Biol. 1:389-404.
- STEWART, W.D.P., FITZGERALD, G.P. & BURRIS, R.H. (1967): *In situ* studies on N_2 fixation using the acetylene reduction technique. - Proc. natn. Acad. Sci. U.S.A. 58:2071-2078.
- STEWART, W.D.P. & PEARSON, H.W. (1970): Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. - Proc. Roy. Soc. Lond. B. 175:293-311.
- TORREY, M.S. & LEE, G.F. (1976): Nitrogen fixation in Lake Mendota, Madison, Wisconsin. - Limnol. Oceanogr. 21:365-378.
- VANDERHOEF, L.N., HUANG, C.-Y., MUSIL, R. & WILLIAMS, J. (1974): Nitrogen fixation (acetylene reduction) by phytoplankton in Green Bay, Lake Michigan, in relation to nutrient concentrations. - Limnol. Oceanogr. 19:119-125.
- VANDERHOEF, L.N., LEIBSON, P.J., MUSIL, R.J., HUANG, C.-Y., FIEHWEG, R.E., WILLIAMS, J.W., WACKWITZ, D.L. & MASON, K.T. (1975): Diurnal variation in algal acetylene reduction (nitrogen fixation) *in situ*. - Plant Physiol. 55:273-276.

- WALSBY, A.E. (1972): Structure and function of gas vacuoles. - *Bacteriol. Rev.* 36:1-32.
- WINBERG, G.G. ET AL. (Eds.) (1971): Symbols, units and conversion factors in studies of fresh water productivity. - I.B.P. Section P.F., I.B.P. Central Office, London.
- WOLK, C.P. (1968): Movement of carbon from vegetative cells to heterocysts in Anabaena cylindrica. - *J. Bacteriol.* 96:2138-2143.
- WOLK, C.P., AUSTIN, S.M., BORTINS, J. & GALONSKY, A. (1974): Autoradiographic localization of ^{13}N after fixation of ^{13}N -labeled nitrogen gas by a heterocyst-forming blue-green alga. - *J. Cell Biol.* 61:440-453.
- WURTSBAUGH, W.A. & HORNE, A.J. (1983): Iron in eutrophic Clear Lake, California: Its importance for algal nitrogen fixation and growth. - *Can. J. Fish. Aquat. Sci.* 40:1419-1429.
- YATES, M.G. & EADY, R.R. (1980): The physiology and regulation of nitrogen fixation. - In: N.S. SUBBA RAO, Ed. Recent advances in biological nitrogen fixation. Holmes and Meier Publishers, New York, pp. 88-120.

Table 1. Morphometric data for the main basin of Lake Trummen before and after restoration (after Gelin & Ripl 1978).

Longitude	14° 50' E	
Latitude	56° 52' N	
Altitude, m.a.s.l.	161.2	
	Before	After
	restoration	restoration
Area, km ²	0.81	0.65
Volume, 10 ⁶ m ³	0.89	1.14
Max. depth, m	2.1	2.5
Mean depth, m	1.1	1.8
Drainage area, km ²	13.1	
Urban, %	25	30
Lakes, %	9	
Theor. retention		
time, months	4	5
Mean annual pre-		
cipitation, mm	645	

Table 2. Multiple regression coefficients for acetylene-reduction rates on water chemistry and heterocystous blue-green algal biomass.

A. Dependent variable: $\text{mmol C}_2\text{H}_4 \text{ m}^{-2} \text{ 24 h}^{-1}$.

	pH	Tot-P	<u>A. lem-</u> <u>mem.</u>	<u>A. vi-</u> <u>guieri</u>	<u>A. spi-</u> <u>roides</u>	<u>A. gra-</u> <u>cile</u> ^a	Tot. het. algae ^a	Constant	F _{tot}	r ²	n
1973			7.227 ⁵			5.696 ⁵	-2.956 ⁵	-0.111	893 ⁵	1.00	17
1974		0.069 ⁵	0.480 ⁴	-1.540 ⁵	1.281 ⁴	-0.632 ⁵		-3.056	64.4 ⁵	0.97	15
1976						1.244 ⁵		-0.971	27.8 ⁵	0.74	12
1978					0.719 ³	0.411 ⁴		0.016	22.1 ⁵	0.82	13
1973-78	1.830 ²						0.318 ¹	-13.47	17.7 ⁵	0.38	61

B. Dependent variable: $\text{max log mol C}_2\text{H}_4 \text{ mg FW}^{-1} \text{ 2 h}^{-1}$.

	t	NO ₃ -N	Sest-N ug mg DW ⁻¹	<u>A. spi-</u> <u>roides</u>	log <u>A.</u> <u>gracile</u>	Constant	F _{tot}	r ²	n
1973	0.080 ⁵					-7.804	61.6 ⁵	0.91	8
1974				-0.536 ²		-6.492	20.8 ²	0.81	7
1978		-0.012 ²				-6.855	17.0 ²	0.74	8
1973-78			0.027		-0.488 ⁴	-8.592	7.54 ²	0.56	15

Significance levels: 1 = $p < 0.05$, 2 = $p < 0.01$, 3 = $p < 0.025$, 4 = $p < 0.005$, 5 = $p < 0.001$.

a The biomass of A. gracile was given the value 0 g FW m^{-2} during late autumn to early spring when heterocysts were not found.

Table 3. Whole-lake volume-weighted mean concentrations of nutrients and phytoplankton, and transparency in Lake Trummen during 15 June - 15 Sept before and after restoration (water-chemical data from Andersson, unpubl., and non-heterocystous algal biomass from Cronberg, 1982).

	1968	1969	1972	1973	1974	1975	1976	1977	1978
Tot-P, $\mu\text{g l}^{-1}$	710	740	83	72	75	96	66	63	63
$\text{PO}_4\text{-P}$, $\mu\text{g l}^{-1}$	145	225	9.8	11	9.5	8.1	8.8	8.4	8.9
Tot-N, mg l^{-1}	5.8	6.9	1.6	1.6	1.7	2.1	1.3	1.2	1.1
$\text{NH}_4\text{-N}$, $\mu\text{g l}^{-1}$	250 ^{1/}	57	22	49	15	15	25	18	23
$\text{NO}_3\text{-N}$, $\mu\text{g l}^{-1}$	8.6	12	5.3	7.6	5.2	2.6	3.5	3.1	5.0
Fe, mg l^{-1}	1.8	2.7	0.72	0.75	0.87	0.92 ^{2/}	0.48	0.69 ^{2/}	0.49 ^{3/}
Sest-P, $\mu\text{g l}^{-1}$	-	-	-	-	85	-	61	-	46 ^{2/}
Sest-P, $\mu\text{g mg DW}^{-1}$	-	-	-	-	4.4	-	4.2	-	2.5 ^{2/}
Sest-N, mg l^{-1}	-	-	-	-	940	-	760	-	870 ^{2/}
Sest-N, $\mu\text{g mg DW}^{-1}$	-	-	-	-	48	-	52	-	46 ^{2/}
Tot-N:Tot-P	8.2	9.3	19	22	22	22	20	19	18
DIN: $\text{PO}_4\text{-P}$	1.8	0.36	2.8	5.2	2.1	2.2	3.2	2.5	3.2
Sest-N:Sest-P	-	-	-	-	11	-	12	-	18 ^{2/}
Tot. phytopl. biomass, mg FW^{-1}	80 ^{4/}	69 ^{4/}	12	8.3	10	16	6.9	6.4	7.2
Heterocyst. algal biomass, mg FW^{-1}	35 ^{4/}	18 ^{4/}	4.7	2.6	5.8	3.5	3.6	1.2	0.3
Nanoplankton biomass, mg FW^{-1}	0.03 ^{4/}	0.44 ^{4/}	2.8	1.8	1.6	11	1.3	1.5	4.1
Transparency, cm	22	17	60	75	62	35	74	78	77

- Not measured.

1/ One measurement.

2/ 22 July - 15 Sept; 0-1 m.

3/ Two measurements.

4/ For calculation cf. Fig. 8.

Table 4. Acetylene reduction and estimated N_2 fixation before and after restoration in Lake Trummen.

	1968 ^{1/}	1969 ^{1/}	1973	1974	1976	1978
mmol C_2H_4 m^{-2} 24 h ⁻¹ , mean ^{2/}	8.2-130	3.0- 17	4.2	1.7	2.8	0.13
mol C_2H_4 m^{-2} year ⁻¹ , total	0.6-9.3	0.3-1.7	0.54	0.26	0.30	0.02
mg N m^{-2} 24 h ⁻¹ , mean ^{2/}	52-800	18-110	27	11	18	0.85
g N m^{-2} year ⁻¹ , total	3.8- 59	1.8- 11	3.4	1.7	1.9	0.12
kg N lake ⁻¹ year ⁻¹ , $\times 10^3$	2.0- 33	1.0-6.1	2.2	1.1	1.3	0.08

1/ Estimated rates; cf. text and Fig. 8.

2/ Mean over N_2 -fixation period.

LEGENDS

Fig. 1. Vertical patterns of planktonic acetylene reduction in Lake Trummen. A. Profiles of temperature (—) and pH (---), and Secchi transparency ($\perp$). B. Acetylene reduction ($\mu\text{mol C}_2\text{H}_4 \text{ l}^{-1} 2 \text{ h}^{-1}$; —) and bio-mass of heterocystous blue-green algae (mg FW l^{-1} ; $\square$). C. Specific acetylene reduction ($\mu\text{mol C}_2\text{H}_4 \text{ mg FW}^{-1} 2 \text{ h}^{-1}$). N.B. Different scales on horizontal axis.

Fig. 2. Concentrations of phosphate ($\bullet$) and dissolved inorganic nitrogen ($\circ$) at 0.2 and 1.5 m water depth during June-Sept 1973-1975 in Lake Trummen. The 45° line shows the position of points of equal concentration at 0.2 and 1.5 m depth. Dashed lines show $10 \mu\text{g l}^{-1}$ difference between concentrations of the two depths.

Fig. 3. Diurnal variation in temperature, oxygen, and pH at 0.2 (—) and 1.5 (---) m water depth in Lake Trummen on 28 and 29 Aug 1973.

Fig. 4. Diurnal variation in whole-lake volume-weighted acetylene reduction (dotted), and insolation at Lake Trummen.

Fig. 5. Vertical variation of acetylene reduction during diurnal studies on 28 (above) and 29 (below) Aug 1973. Arithmetic mean, and ranges from triplicate samples (horizontal bars).

Fig. 6. Planktonic acetylene reduction (arithmetic mean, and ranges from triplicate samples) and dissolved inorganic nitrogen; seston nitrogen; seston phosphorus and phosphate; and biomass of total phytoplankton assemblage ($\bullet-\bullet$), heterocystous blue-green algae ($\bullet-\bullet$), and *Aphanothece clathrata* ($\circ-\circ$). Plankton maxima in 1976 expressed in g FW m^{-2} .

Fig. 7. Development of biomass of four species of heterocystous blue-green algae in Lake Trummen after restoration. Zero values also denoted.

Fig. 8. Dissolved inorganic nitrogen, phosphate, and biomass of heterocystous blue-green algae in Lake Trummen before restoration. Estimated N_2 -fixation periods denoted (■). Nutrient data from Andersson (unpubl.) and biomass from Cronberg (1982). Before 19 June 1968, ammonium was not analyzed. On most sampling dates winds were hard, and biomass of 0.2 m was taken as representative

for the whole lake volume. On 30 May 1968 weather was calm, and total algal biomass was considered to be accumulated at 0-0.5 m water depth.

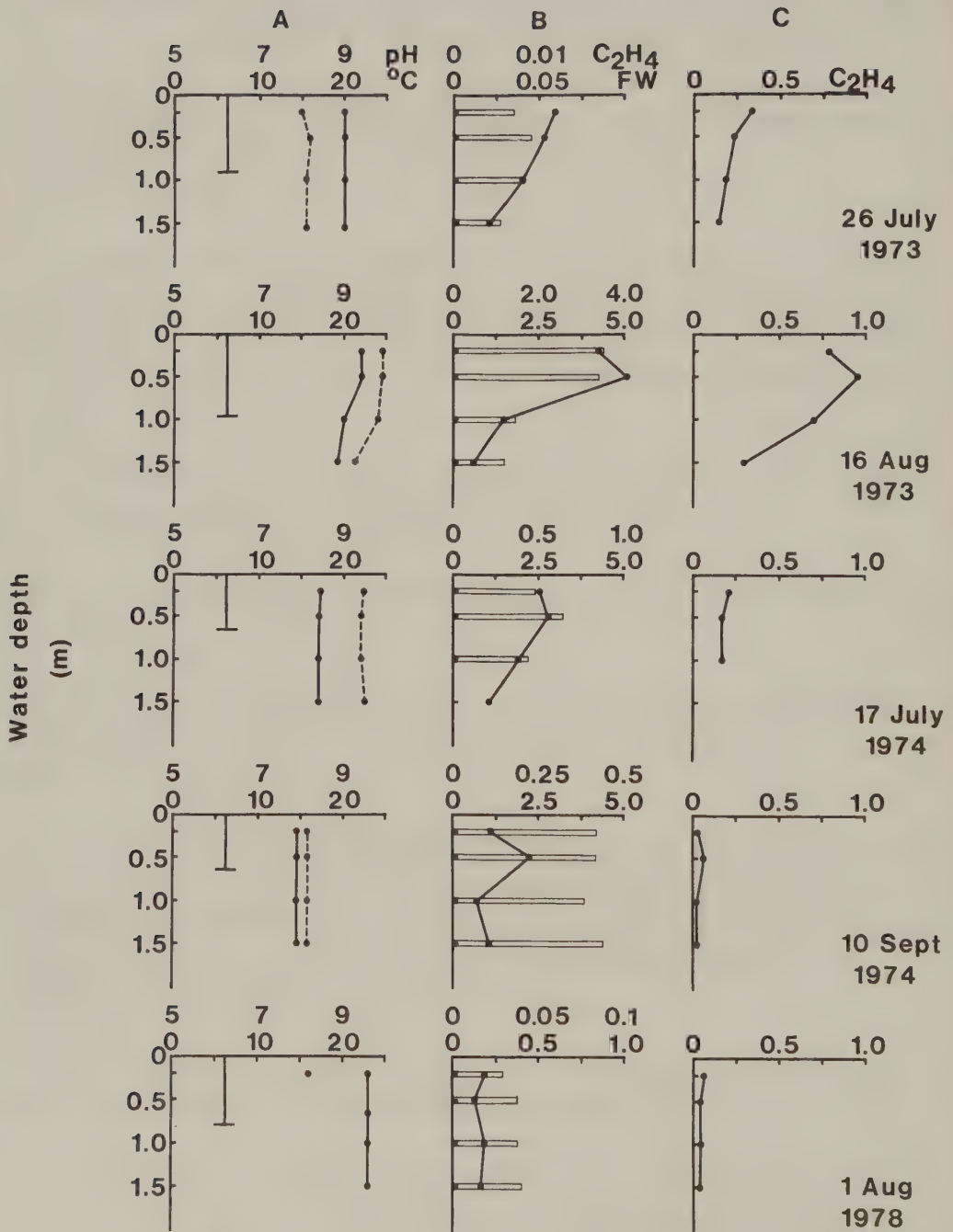


Fig. 1.

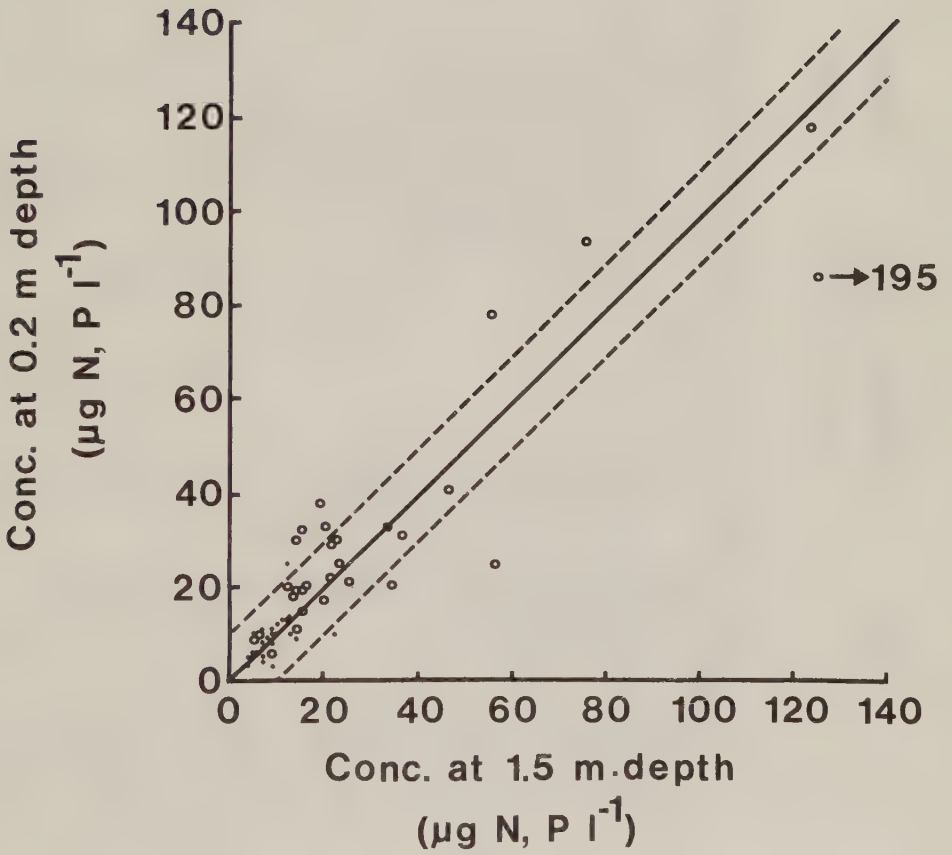


Fig. 2.

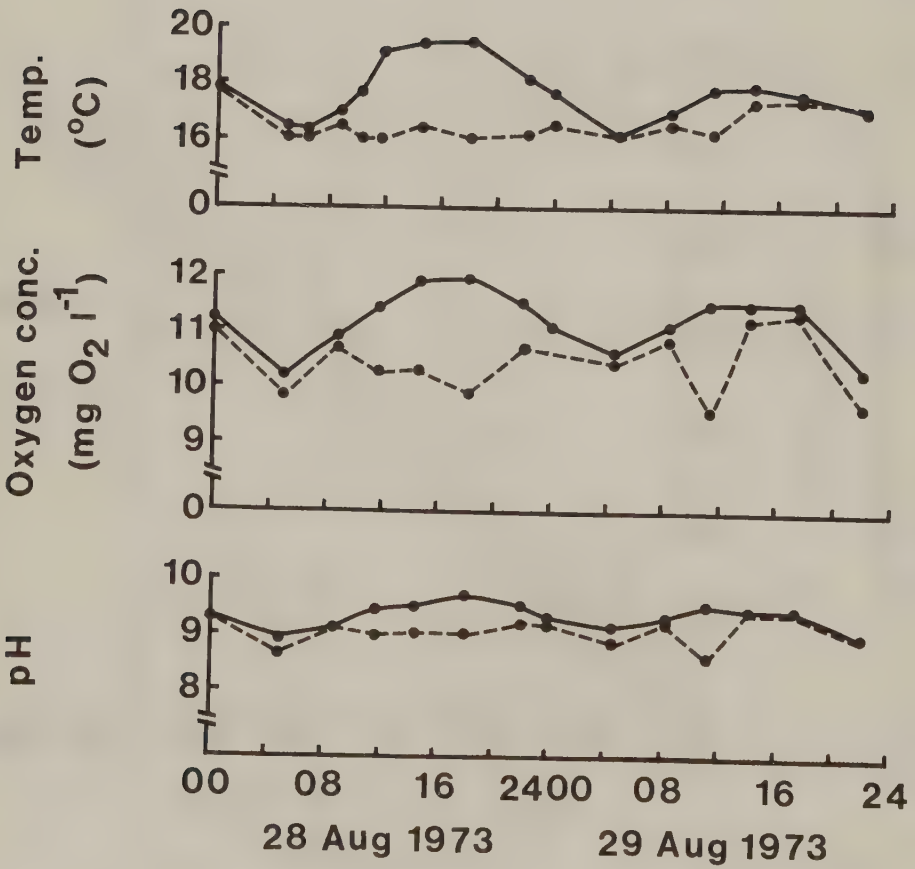


Fig. 3.

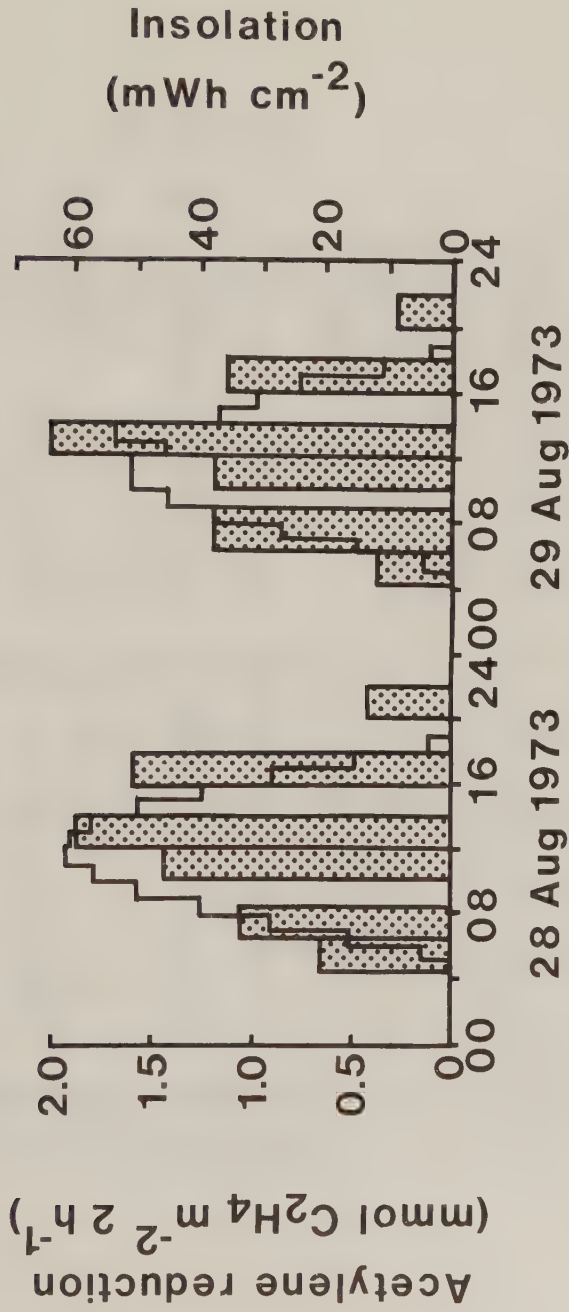


Fig. 4.

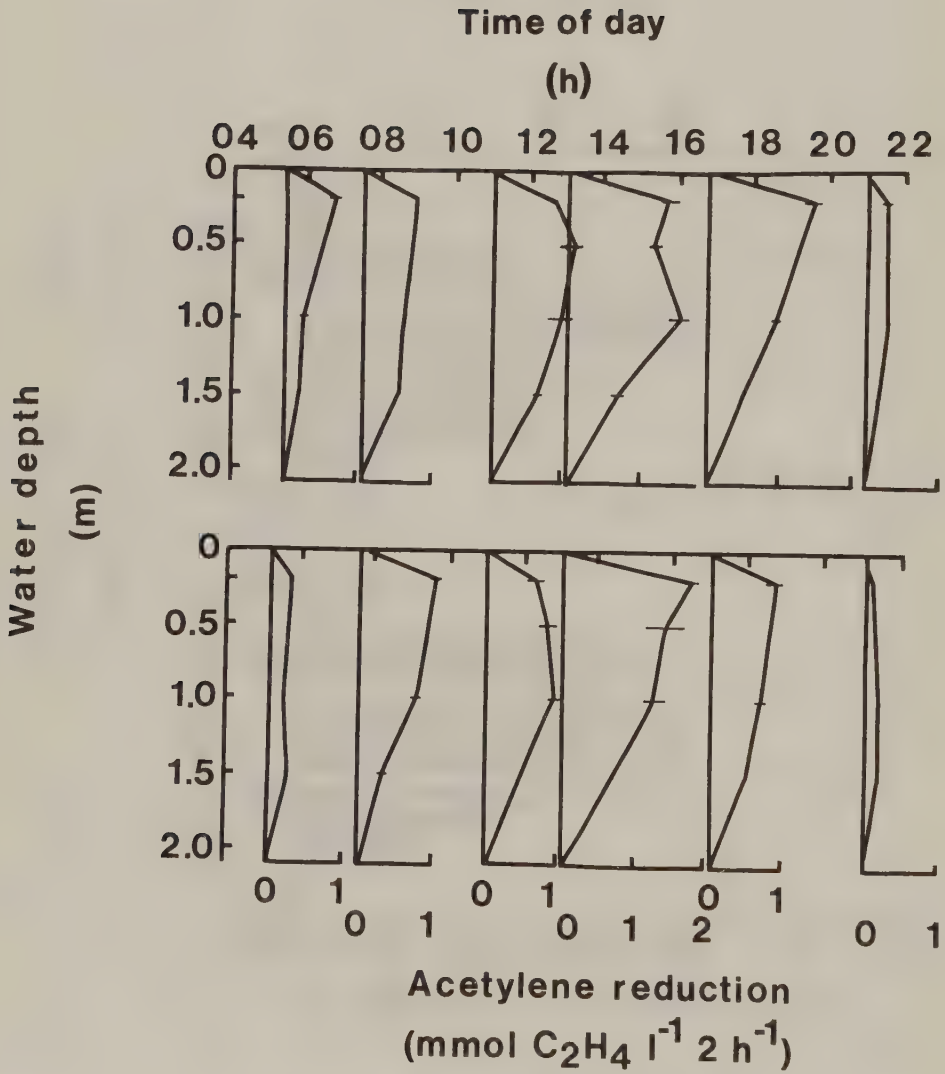


Fig. 5.

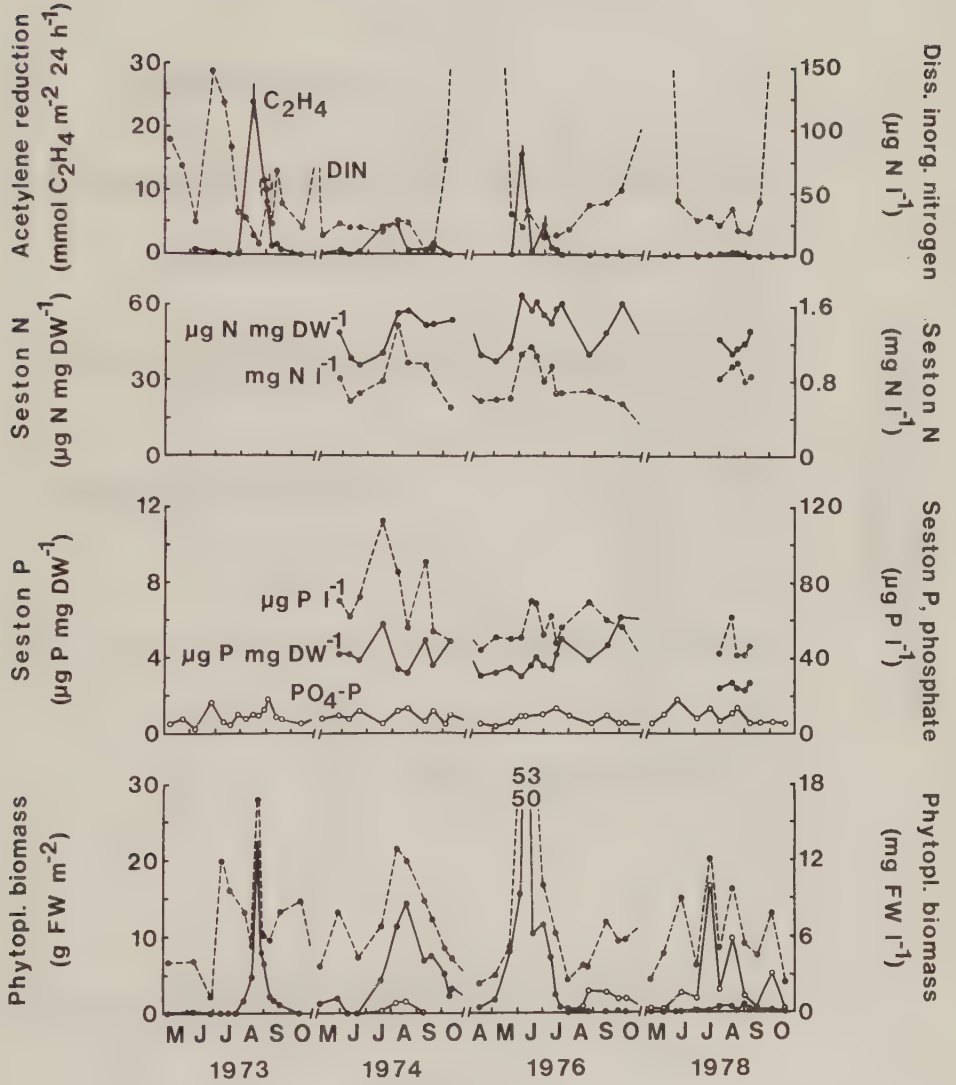


Fig. 6.

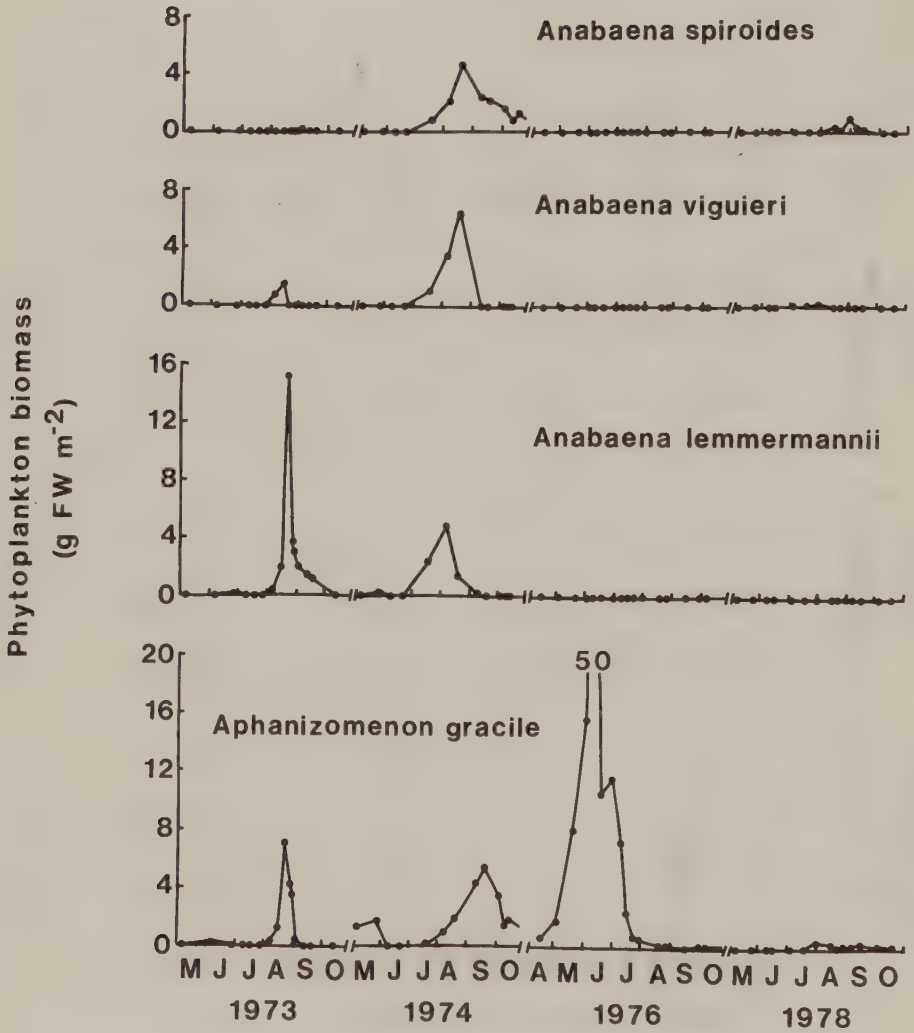


Fig. 7.

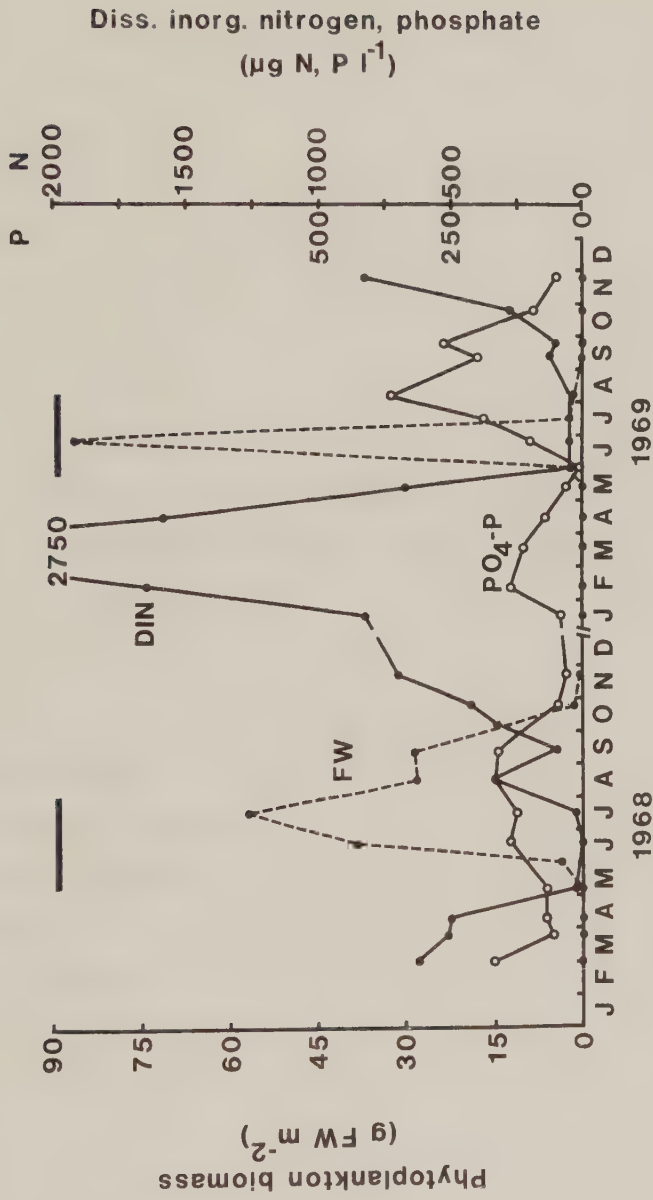


Fig. 8.

Does N_2 fixation meet the nitrogen requirements of heterocystous blue-green algae in shallow eutrophic lakes?

Lars Leonardson
Institute of Limnology
University of Lund
Box 3060
S- 220 03 LUND
Sweden

ABSTRACT

Phytoplankton net carbon uptake and nitrogen fixation were studied in two shallow, eutrophic lakes in South Sweden. Ranges of diurnal net carbon uptake were estimated by subtracting 24-h respiration rates corresponding to 5-20% of $P_{\max}$, respectively, from daytime carbon uptake values. Total nitrogen requirement of the phytoplankton assemblage was determined from the diurnal net carbon uptake, assuming a phytoplankton C:N ratio of 9.5:1. Nitrogen supplied by nitrogen fixation only occasionally corresponded to the demands of the total phytoplankton assemblage. When heterocystous algae made up a substantial proportion ($\geq 10\%$) of the total phytoplankton biomass, nitrogen fixation could meet the requirements of heterocystous blue-green algae on c. 50% of the sampling occasions. Nitrogen deficiencies in heterocystous algae were most probably balanced by the simultaneous or sequential assimilation of dissolved inorganic nitrogen. It was concluded that uptake of ammonium or nitrate, regenerated from lake seston and sediment, is the main process by which growth of phytoplankton is maintained during summer in the lake ecosystems studied.

INTRODUCTION

Contributions by blue-green algal nitrogen (N_2) fixation to the nitrogen budget of eutrophic lakes have often been considered necessary for supporting high phytoplankton biomass and primary production during summer (e.g. Granhall and Lundgren 1971, Horne and Viner 1971, Horne and Goldman 1972, Vanderhoef et al. 1974, Leonardson and Bengtsson 1978, Ashton 1981). However, on most occasions non-nitrogen-fixing phytoplankton species and nitrogen-fixing species coexist throughout the growth period of N_2 fixers. Dugdale and Goering (1967), working in oligotrophic ocean areas, suggested that generation of new nitrogen, e.g. by nitrogen fixation, is merely a supplement to internal N turnover in the euphotic zone, and that demands of non-nitrogen-fixing species are met to a large extent by recycled nitrogen. The question arises: If there is a high rate of mineralization of organic nitrogen, to what extent do nitrogen-fixing algae utilize dissolved nitrogen compounds which are energetically cheaper nitrogen sources than molecular nitrogen?

Although primary production and nitrogen fixation are measures frequently used to describe metabolic activity in aquatic environments, only limited information, primarily marine, is available on the fraction of phytoplankton nitrogen requirements covered by nitrogen fixation. Nitrogen fixation was felt to supply less than 20% of the immediate nitrogen demand for phytoplankton primary production (Carpenter and Price 1977,

Mague et al. 1977, Lindahl et al. 1980), and great variations were found in the supply by nitrogen fixation to heterocystous algae.

Carpenter and McCarthy (1975) showed that concentrations of ammonium, nitrate, and urea in the Sargasso Sea were too low to be utilized by Oscillatoria thiebautii and concluded that this species had to rely on nitrogen supply through nitrogen fixation. Similarly, Mague et al. (1977) found that 100% of the nitrogen requirement of isolated Trichodesmium (Oscillatoria) colonies was furnished by nitrogen fixation. Other estimates of nitrogen supply to Oscillatoria indicated that nitrogen fixation supplied 40-140% of the demands of this species in the Caribbean and Sargasso Seas (Carpenter and Price 1977). Short-term studies on individual colonies revealed that between 12 and 1100% of their immediate requirement was met by nitrogen fixation. No estimates of this sort have been reported for freshwater phytoplankton.

In the current investigation the relative importance of nitrogen fixation and uptake of dissolved inorganic nitrogen for heterocystous algae and the whole phytoplankton assemblage was studied in two shallow, eutrophic lakes containing high phytoplankton biomasses.

MATERIALS AND METHODS

Lakes studied

The shallow Lakes Trummen and Södra Bergundasjön, situated in the South Swedish oligotrophic bedrock area, were

eutrophicated by sewage effluents during the first half of the 20th century. Restoration of Lake Trummen during 1970-71 resulted in less eutrophic conditions, and predominance of diatoms, nitrogen-fixing Anabaena spp. and Aphanizomenon gracile Lemm., and undetermined μ -algae in summer phytoplankton (Cronberg 1982, Leonardson in prep). Diversion of sewage from Lake Södra Bergundasjön in 1974 lowered the lake water concentration of dissolved inorganic nitrogen during spring and summer, although phosphorus levels remained high. The phytoplankton assemblage was characterized by summer blooms of Anabaena spiroides var. crassa Lemm., and Microcystis spp. (Bengtsson 1975, Leonardson and Bengtsson 1978). Some lake data are given in Tab. 1.

Tab. 1.

Phytoplankton primary production

Phytoplankton primary production was estimated with the ^{14}C -productivity method (Steemann Nielsen 1952) in Lake Trummen (Gelin and Riip 1978) and Lake Södra Bergundasjön (Leonardson and Bengtsson 1978). In Lake Trummen water samples from six or seven (0.1-1.5/2.0 m) depths were exposed on the sampling levels during 1.5 h from true noon during 1973 and 1974, and from true noon to sunset during 1973. In Lake Södra Bergundasjön primary production measurements were made using mixed water samples from 0-1 m exposed in bottles at six to seven (0.1-1.0/1.5 m) depths from true noon to sunset during 1975 and 1976. Dark bottles were incubated at 0.2 m and at the lowest sampling depth. Daily production rates for

the different water depths were estimated by multiplying half-day exposure rates by 2, and 1.5 h exposure rates by the ratio of daily insolation:insolation during incubation period.

Respiration rates must be considered in the calculation of diurnal (24 h) net carbon uptake. For both lakes, low and high limits on respiration of 5 and 20% of $P_{\max}$ (Harris 1978) were used, assuming constant respiration during a 24-h day, and considering the vertical distribution of phytoplankton. Half-day ^{14}C uptake was assumed to measure net carbon assimilation in the euphotic zone during light hours (cfr. Devol and Packard 1978). Night-time respiration was then calculated for the "euphotic zone", and 24-h respiration for the aphotic zone. Alternatively, for Lake Södra Bergundasjön night-time respiration was also taken to be 30-50% of daily production in the euphotic zone (Devol and Packard 1978). Diurnal net carbon uptake was estimated by subtracting the obtained respiration value from daily primary production. Results were expressed as whole-lake volume-weighted net primary production per day per unit lake area.

Nitrogen fixation

Water for nitrogen-fixation (acetylene-reduction) studies was sampled at four depths (0.2-1.5 m, and 0.2-4.5 m, respectively) in Lakes Trummen and Södra Bergundasjön. Experimental procedures for nitrogen-fixation measurements and ethylene analysis were according to Leonard-

son (1983). In both lakes incubations were made at the depths from which organisms were sampled. In Lake Trummen heterocystous blue-green algae were concentrated 4-100X by plankton nets, except on 19 Aug and 10 Sept 1974. Acetylene-reduction rates of concentrated samples were corrected for by the ratio of cell volume of heterocystous algae in unconcentrated samples:cell volume of heterocystous algae in concentrated samples. Concentrating phytoplankton in Lake Trummen has been shown not to influence specific acetylene-reduction rates (Leonardson 1983). In Lake Södra Bergundasjön algae were not concentrated. Six experiments with unconcentrated algal suspensions from Lakes Trummen and Södra Bergundasjön during 1976-1977 showed that the use of 1.0 ml acetylene in the incubations ($0.07 \text{ ml C}_2\text{H}_2 \text{ ml H}_2\text{O}^{-1}$; Flett et al. 1976) on average resulted in underestimates of acetylene reduction by 40% (range 20-65%), compared to additions of 2.0 ml acetylene (after evacuation of 1.0 ml air). Additions of 3.0 and 5.0 ml acetylene (after evacuation of 2.0 and 4.0 ml air) inhibited the nitrogenase activity (cfr. Hardy et al. 1968). Thus, all N_2 -fixation data were corrected by the factor 1.4. Diurnal rates of acetylene reduction were calculated by using the ratio of daily insolation:incubation-period insolation corrected for nocturnal acetylene reduction (Leonardson in prep). The molar ratio 4.4:1 was used to convert acetylene reduction into N_2 fixation (Peterson and Burris 1976). Results are presented as whole-lake volume-weighted nitrogen fixation per day per unit lake area.

Phytoplankton biomass

Contents of incubation tubes for acetylene-reduction measurements were used for counting blue-green algae from 3-4 depths with the inverted microscope technique (cfr. Leonardson 1983). Since no vertical stratification of algae occurred in Lake Trummen (Gelin and Rippl 1978, Cronberg 1982), and the water of Lake Södra Bergundasjön was only weakly thermally stratified on few occasions, dominating non-blue-green algal species were counted at one level (0.2 m and 0-1 m, in Lakes Trummen and Södra Bergundasjön, respectively). In determining the fresh weight of algae from cell volumes, algal specific weight was assumed to be 1.0. For Lake Trummen, published data on fresh weight of non-blue-green algae were used when available (Cronberg 1982). Results are presented as whole-lake volume-weighted fresh weight per unit lake area.

Carbon and nitrogen in seston, and phytoplankton nitrogen requirements

During 1974 and 1976 seston was collected in Lakes Trummen and Södra Bergundasjön from 0-1 m water depth. Seston dry weight was determined on Whatman GF/C filters dried at 105 °C. Filters were stored frozen until analysis for carbon and nitrogen using a Carlo Erba Elemental Analyzer, model 1106. Seston C:N ratios were expressed on a weight basis.

Phytoplankton nitrogen requirements were estimated by dividing diurnal net carbon uptake per unit lake area by a C:N ratio of moderately starved phytoplankton

(C:N=9.5:1, derived from Healey 1975). It was assumed that the nitrogen demand of heterocystous algae was proportional to the percentage of heterocystous algae in the total phytoplankton biomass.

RESULTS

Net carbon uptake rates estimated using different rates of respiration showed similar patterns during summer in Lakes Trummen and Södra Bergundasjön (Fig. 1 and 2). However, since use of respiration rates equal to 20% of $P_{\max}$, as well as night-time respiration rates of 30-50% of daily production in the euphotic zone (Devol and Packard 1978), resulted in negative net carbon uptake rates in periods with increasing phytoplankton biomass or high nitrogen fixation in Lake Trummen 1974 and Lake Södra Bergundasjön 1975, these estimates seem less realistic. Comparison of daily uptake rates from half-day and 1.5-h exposures in the euphotic zone of Lake Trummen during 1973 indicated respiration rates less than 5% of $P_{\max}$. In Lake Trummen changes in phytoplankton biomass were strongly correlated to changes in net carbon uptake rates, while such patterns usually did not appear in Lake Södra Bergundasjön (Fig. 1 and 2). The development of nitrogen-fixation rates did not follow that of net carbon uptake in either lake, although maximum N_2 fixation appeared on sampling dates when net carbon uptake rates (estimated at low respiration rates) were at or near maximum.

During summer 1973 Anabaena lemmermannii P. Richt., A. viguieri Denis et Frémy and Aphanizomenon gracile made up the nitrogen-fixing fraction of the diverse phy-

toplankton assemblage in Lake Trummen, while Anabaena spiroides var. spiroides Kleb. also was present during 1974. Quantitatively important non-heterocystous algae were Microcystis aeruginosa Kütz., Synechococcus vantieghemi (Pringsh.) Bourr., Pediastrum spp., Staurodesmus spp., Cyclotella spp., Stephanodiscus spp. and Melosira spp.. Especially during 1973, taxonomically undetermined micro-algae were common (Cronberg 1982). In Lake Södra Bergundasjön Anabaena spiroides var. crassa, the sole nitrogen fixer, and Microcystis spp. dominated the phytoplankton assemblage during summers, while green algae (Scenedesmus spp., Pediastrum spp.) and diatoms (Melosira spp., Asterionella formosa Hass., Stephanodiscus hantzschii Grun.) were less frequent.

During the periods May-September 1974 and 1976, mean C:N ratios of epilimnetic seston varied between 5.5:1 and 9.5:1 in Lake Trummen, and between 4.5:1 and 7.1:1

Fig. 3. in Lake Södra Bergundasjön (Fig. 3). The maximum C:N ratio found, 9.5:1, agreed with phytoplankton C:N ratios from the literature, indicating moderate nutrient deficiency (Healey 1975). Thus, in order not to overestimate phytoplankton nitrogen demands when evaluating the importance of N_2 fixation, a respiration rate of 15% of P_{max} and a C:N ratio of 9.5:1 were used to calculate diurnal net C uptake and the corresponding N demand.

Nitrogen requirements of heterocystous blue-green algae making up 10% or more of the phytoplankton fresh weight were totally supported by nitrogen fixation on 5 out of 9 occasions in Lake Trummen, and on 2 out of 6 occasions in Lake Södra Bergundasjön, provided that C:N

ratios of 9.5:1 and respiration rates corresponding to 15% of $P_{\max}$ are accurate assumptions (Fig. 4). In addition, on two occasions in Lake Trummen, when the proportions of heterocystous algae in the total biomass were only 10 and 14%, 80 and 85% of their nitrogen demands were supplied by nitrogen fixation. On the occasions when heterocystous algae were $\geq 10\%$ of the total phytoplankton biomass, the N demands (amounting to 7-105, and 0-245 $\text{mg N m}^{-2} 24 \text{ h}^{-1}$, respectively) of the total phytoplankton assemblages were supported to 100% or more on 3 and 1 occasions in Lakes Trummen and Södra Bergundasjön (Fig. 4). In the latter lake this occurred on 16 Sept 1975, when estimated net carbon uptake was negative, but N_2 fixation amounted to 14 $\text{mg N m}^{-2} 24 \text{ h}^{-1}$. Except for the occasion of negative net carbon uptake, the proportions of total phytoplankton nitrogen requirements furnished by N_2 fixation was 7-150%, and 5-82%, in the two lakes. Consequently, estimated diurnal amounts of nitrogen assimilated from nitrogen sources other than dinitrogen varied between 0 and 76, and 14 and 200 mg N m^{-2} (corresponding to 0-45, and 7-100 $\mu\text{g N l}^{-1}$), respectively, in the two lakes (Tab. 2).

Tab. 2.

When calculations were based on a lower C:N ratio or lower respiration rate, the inequality between nitrogen requirement and N_2 fixation increased; e.g. at a respiration rate corresponding to 5% of $P_{\max}$ N requirements were supported on only 3 of the sampling dates in Lake Trummen. On the contrary, at a respiration rate of 20% of $P_{\max}$ nitrogen fixation supported the N demands of heterocystous blue-green algae on 8 out of 9 occasions in Lake Trummen, but only on 3 out of 6 occasions in

Lake Södra Bergundasjön. On these conditions total net carbon uptake was sometimes negative (Fig. 1 and 2), and the corresponding percentage of nitrogen supplied by nitrogen fixation was infinite.

Whole-lake volume-weighted concentrations of dissolved inorganic nitrogen were less than $50 \mu\text{g l}^{-1}$ during the periods of active N_2 fixation in Lake Trummen (Fig. 1). Mean concentrations in Lake Södra Bergundasjön were not as low as in Lake Trummen, and reached very high values during the start and end of N_2 -fixation periods (Fig. 2).

DISCUSSION

In the eutrophic lakes of this study, nitrogen fixation on several occasions met the immediate nitrogen requirements of heterocystous blue-green algae. Furthermore, on some of these dates the nitrogen demands of the total phytoplankton assemblages could also have been met by N_2 fixation. No general patterns were found between percent nitrogen supported by nitrogen fixation and net carbon uptake rate or development phase of heterocystous algae (Fig. 1 and 2; also cfr. Fig. 1 in Leonardson and Bengtsson 1978, and Fig. 6 in Leonardson in prep.). However, low supply by nitrogen fixation was more frequent in Lake Södra Bergundasjön than in Lake Trummen, which was probably due to inhibition by higher availability of dissolved inorganic nitrogen (Fig. 1 and 2; cfr. Stewart et al. 1968, Ogawa and Carr 1969, Murry and Benemann 1979). The source of this nitrogen would have primarily been release from the sediment and from decay-

ing plankton organisms, and only to a small extent nitrogen transport into the lake from the watershed (cfr. Bengtsson 1978, and Tab. 2).

Contrary to these findings in freshwaters, nitrogen fixation in the oceans (Carpenter and Price 1977, Mague et al. 1977) and in the Baltic (Lindahl et al. 1980) did not meet the N requirements of the total phytoplankton on any occasion. However, the authors cited did not consider respiration in carbon uptake calculations, so their estimates of percent nitrogen supplied by nitrogen fixation were probably low. As in heterocystous freshwater algae, N_2 fixation by marine nitrogen-fixing algae varied between very low values and full satisfaction of N demands (Carpenter and McCarthy 1975, Carpenter and Price 1977, Mague et al. 1977).

Low supply of nitrogen by nitrogen fixation, in relation to the requirements of heterocystous blue-green algae, suggests (1) that nitrogen deficiencies might be compensated for by nitrogen fixation or uptake of dissolved inorganic nitrogen on days immediately before or after the sampling dates, or (2) that supplementary uptake of ammonium and/or nitrate occurs simultaneously with nitrogen fixation. The first alternative is supported by the very high supply by N_2 fixation found on a few occasions in Lakes Trummen and Södra Bergundasjön. However, these estimates were based on a high C:N ratio (moderate nitrogen starvation, Healey 1975), and on high respiration rates (15-20% of P_{max}), both of which might lead to an over-

estimate of the importance of nitrogen fixation. Gerloff and Skoog (1957), Healey (1975), and Rhee (1978) found evidence for luxury consumption of dissolved inorganic nitrogen in phytoplankton. Murphy and Brownlee (1981) reported that Aphanizomenon flos-aquae and Microcystis aeruginosa were able to rapidly increase their uptake velocity for ammonium, and strongly compete for occasional high fluxes of this ion. The authors suggested that the assimilated NH_4 was immediately used for growth, or stored and used under nutrient-deficient conditions. However, Fogg (1971) discussed the occurrence of intensive excretion of nitrogenous compounds from actively growing N_2 -fixing organisms.

Using ^{15}N -tracer techniques, Dugdale and Dugdale (1965), and Liao and Lean (1978) demonstrated concurrent uptake of ammonium, nitrate and molecular nitrogen by freshwater phytoplankton assemblages. Since the assemblages consisted of several algal groups, it was usually not possible to ascribe the uptake of different nitrogen fractions to a certain algal group or species. However, measurements performed by Billaud (1968) on a phytoplankton assemblage totally dominated by Anabaena flos-aquae indicated that this species was able to assimilate ammonium and nitrate simultaneously with nitrogen-fixation activity.

Laboratory experiments have shown that ammonium and nitrate inhibit the formation of new heterocysts, while permitting continued functioning of existing nitrogenase

(Stewart et al. 1968, Murry and Benemann 1979). Nitrate did not affect nitrogen fixation as severely as ammonium (Ogawa and Carr 1969). These studies were usually performed at high concentrations of ammonium and nitrate, so conclusions cannot be applied uncritically to lake conditions where concentrations and availability of dissolved inorganic nitrogen are low. Brownlee and Murphy (1983) reported seasonal peak rates of nitrogenase activity when there was $100\text{--}300 \mu\text{g NH}_4\text{-N l}^{-1}$ in the lake water. No evidence for short-term ammonium inhibition of N_2 fixation was found. Similarly, the nitrogen fixation in an epilithic periphyton assemblage did not decline as a result of moderate in-situ additions of ammonium and nitrate (Reuter et al. 1983). Moreover, Goering (1962, cited by Dugdale and Dugdale 1965), and Goering and Neess (1964) found that low concentrations of ammonium occasionally enhanced nitrogen fixation in lakes. In laboratory batch cultures, exponentially growing Anabaena cylindrica assimilated ammonium at concentrations of $400 \mu\text{g N l}^{-1}$ and less during short periods and simultaneously increased nitrogen-fixation rates (Leonardson unpubl.).

Consequently, both field experiences and literature data suggest that heterocystous blue-green algae in eutrophic freshwaters assimilate dissolved inorganic nitrogen, either simultaneously or sequentially, during N_2 -fixation periods.

The supply of nitrogen to phytoplankton by internal sources was estimated for Lake Södra Bergundasjön during 1975 and 1976. Due to low import of nitrogen during sum-

mer the high phytoplankton demands must have been furnished by lake-internal sources (Tab. 2). In 1976 high lake-water concentrations of ammonium and nitrate were depleted, while in 1975 these nutrients occurred at low and stable levels until September (Fig. 2). Based on sediment gross release data (Bengtsson 1978, and pers. comm.), it was estimated that sediment release could have made up 40% and more of the internal nitrogen demand. However, gross release figures probably overestimate the nitrogen flux from the sediment to phytoplankton, because part of the released ammonium might be made inaccessible to phytoplankton by coupled nitrification-denitrification processes at the sediment surface (Ripl and Lindmark 1979). On the other hand, sediment net release values, ranging between 0 and $70 \text{ mg N m}^{-2} 24 \text{ h}^{-1}$ (Bengtsson 1978), underestimate the availability of nitrogen to phytoplankton. The corresponding estimated supply of nitrogen by seston turnover (in Tab. 2) must therefore be considered to be too low. Using the minimum summer seston turnover rate found by Bloesch et al. (1977) in two meso- to eutrophic Swiss lakes (8%), resulted in potential nitrogen supply rates of $220\text{--}590 \text{ mg N m}^{-2} 24 \text{ h}^{-1}$. This indicates that the total internal nitrogen demand could have been met by degradation of seston and nutrient release within the pelagial of Lake Södra Bergundasjön.

In conclusion, this paper shows (1) that even though phytoplankton N demand was probably underestimated (due to a choice of high respiration rate and high C:N ratio), nitrogen fixation by heterocystous blue-green algae met

the requirements of themselves only on c. 50% of the sampling occasions, and only occasionally corresponded to the N demands of the total phytoplankton assemblage, and (2) that internally regenerated nitrogen was important for the maintenance and growth of heterocystous and non-heterocystous algae during summer. At the ecosystem level, nitrogen fixation then functions as a supplementary mechanism that compensates for nitrogen deficiency in the euphotic zone, or as a process which occasionally allows rapid increase of phytoplankton biomass (cfr. Dugdale and Goering 1967). At the population level nitrogen fixation is a valuable evolutionary trait, allowing heterocystous algae to outcompete other algae during periods with low availability of nitrate and ammonium.

ACKNOWLEDGMENTS

I thank Prof Sven Björk, Drs G. Andersson, M.F. Convey, W. Granéli and A. Södergren, and Mr G. Gahnström for valuable comments on the manuscript. Mr J. Jonasson helped with field work and GC analyses, and Dr G. Cronberg, Miss A. Fritzon and Mr L. Tranvik did the plankton analyses. Dr C. Gelin and Mr L. Bengtsson kindly supplied primary-productivity data from Lakes Trummen and Södra Bergundasjön, respectively. I am also grateful to Drs G. and I. Ahlgren allowing me access to the Carlo Erba Elemental Analyzer at the Institute of Limnology in Uppsala. Financial support was given by The National Swedish Environment Protection Board and The Royal Swedish Academy of Science.

REFERENCES

- Ashton, P.J. 1981. Nitrogen fixation and the nitrogen budget of a eutrophic impoundment. *Wat. Res.* 15:823-833.
- Axler, R.P., G.W. Redfield, and C.R. Goldman. 1981. The importance of regenerated nitrogen to phytoplankton productivity in a subalpine lake. *Ecology* 62:345-354.
- Bengtsson, L. 1975. Phosphorus release from a highly eutrophic lake sediment. *Verh. Internat. Verein. Limnol.* 19:1107-1116.
- Bengtsson, L. 1978. Effects of sewage diversion in Lake Södra Bergundasjön. I. Nitrogen and phosphorus budgets. *Vatten* 34:2-9.
- Billaud, V.A. 1968. Nitrogen fixation and the utilization of other inorganic nitrogen sources in a subarctic lake. *J. Fish. Res. Board Can.* 25:2101-2110.
- Bloesch, J., P. Stadelmann, and H. Bührer. 1977. Primary production, mineralization, and sedimentation in the euphotic zone of two Swiss lakes. *Limnol. Oceanogr.* 22:511-526.
- Brownlee, B.G. and T.P. Murphy. 1983. Nitrogen fixation and phosphorus turnover in a hypertrophic prairie lake. *Can. J. Fish. Aquat. Sci.* 40:1853-1860.
- Carpenter, E.J., and J.J. McCarthy. 1975. Nitrogen fixation and uptake of combined nitrogenous nutrients by Oscillatoria (Trichodesmium) thiebautii in the western Sargasso Sea. *Limnol. Oceanogr.* 20:389-401.
- Carpenter, E.J., and C.C. Price IV. 1977. Nitrogen fixa-

tion, distribution, and production of Oscillatoria (Trichodesmium) spp. in the western Sargasso and Caribbean Seas. *Limnol. Oceanogr.* 22:60-72.

Cronberg, G. 1982. Phytoplankton changes in Lake Trummen induced by restoration. Long-term whole-lake studies and food-web experiments. *Folia Limnologica Scandinavica* 18. 119 p.

Devol, A.H., and T.T. Packard. 1978. Seasonal changes in respiratory enzyme activity and productivity in Lake Washington microplankton. *Limnol. Oceanogr.* 23:104-111.

Dugdale, V.A., and R.C. Dugdale. 1965. Nitrogen metabolism in lakes. III. Tracer studies of the assimilation of inorganic nitrogen sources. *Limnol. Oceanogr.* 10:53-57.

Dugdale, R.C., and J.J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* 12:196-207.

Fogg, G.E. 1971. Extracellular products of algae in freshwater. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 5:1-25.

Gelin, C., and W. Ripl. 1978. Nutrient decrease and response of various phytoplankton size fractions following the restoration of Lake Trummen, Sweden. *Arch. Hydrobiol.* 81:339-367.

Gerloff, G.C., and F. Skoog. 1957. Nitrogen as a limiting factor for the growth of Microcystis aeruginosa in southern Wisconsin lakes. *Ecology* 38:556-561.

Goering, J.J. 1962. Studies of nitrogen fixation in natu-

- ral fresh waters. Ph. D. Thesis. Univ. Wisconsin, Madison. 133 p.
- Goering, J.J., and J.C. Neess. 1964. Nitrogen fixation in two Wisconsin lakes. *Limnol. Oceanogr.* 9:530-539.
- Granhall, U., and A. Lundgren. 1971. Nitrogen fixation in Lake Erken. *Limnol. Oceanogr.* 16:711-719.
- Hardy, R.W.F., R.D. Holsten, E.K. Jackson, and R.C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiol.* 43:1185-1207.
- Harris, G.P. 1978. Photosynthesis, productivity and growth: The physiological ecology of phytoplankton. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 10:I-IV, 1-171.
- Healey, F.P. 1975. Physiological indicators of nutrient deficiency in algae. *Fish. Mar. Serv. Tech. Rep.* 585, 30 p.
- Horne, A.J., and C.R. Goldman. 1972. Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. *Limnol. Oceanogr.* 17:678-692.
- Horne, A.J., and A.B. Viner. 1971. Nitrogen fixation and its significance in tropical Lake George, Uganda. *Nature* 232:417-418.
- Leonardson, L. 1983. Effects of concentrating phytoplankton on the acetylene-reduction assay for nitrogenase activity. *Freshwat. Biol.* 13:265-274.
- Leonardson, L. In prep. Blue-green algal nitrogen fixation before and after restoration of Lake Trummen, Sweden.

- Leonardson, L., and L. Bengtsson. 1978. Effects of sewage diversion in Lake Södra Bergundasjön. II. Phytoplankton changes and the role of nitrogen fixation. Verh. Internat. Verein. Limnol. 20:2701-2707.
- Liao, C.F.-H., and D.R.S. Lean. 1978. Nitrogen transformations within the trophogenic zone of lakes. J. Fish. Res. Board Can. 35:1102-1108.
- Lindahl, G., K. Wallström, and G. Brattberg. 1980. Short-term variations in nitrogen fixation in a coastal area of the Northern Baltic. Arch. Hydrobiol. 89:88-100.
- Mague, T.H., F.C. Mague, and O. Holm-Hansen. 1977. Physiology and chemical composition of nitrogen-fixing phytoplankton in the Central North Pacific Ocean. Mar. Biol. 41:213-227.
- Murphy, T.P. and B.G. Brownlee. 1981. Blue-green algal ammonia uptake in hypertrophic prairie lakes. Can. J. Fish. Aquat. Sci. 38:1040-1044.
- Murry, M.A. and J.R. Benemann. 1979. Nitrogenase regulation in Anabaena cylindrica. Pl. Cell Physiol., Tokyo 20:1391-1401.
- Ogawa, R.E., and J.F. Carr. 1969. The influence of nitrogen on heterocyst production in blue-green algae. Limnol. Oceanogr. 14:342-351.
- Peterson, R.B., and R.H. Burris. 1976. Conversion of acetylene reduction rates to nitrogen fixation rates in natural populations of blue-green algae. Anal. Biochem. 73:404-410.
- Reuter, J.E., S.L. Loeb, R.P. Axler, R.G. Carlton and

- C.R. Goldman. 1983. Transformations of nitrogen following an epilimnetic N-fertilization in Castle Lake, CA: periphyton responses. Abstracts. XXIIInd Congress of the International Association of Limnology, Lyon, France, 21-28 Aug 1983.
- Rhee, G.-Y. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.* 23:10-25.
- Ripl, W., and G. Lindmark. 1979. The impact of algae and nutrient composition on sediment exchange dynamics. *Arch. Hydrobiol.* 86:45-65.
- Steemann Nielsen, E. 1952. The use of radioactive carbon (C^{14}) for measuring organic production in the sea. *J. Cons. perm. int. Explor. Mer* 18:117-140.
- Stewart, W.D.P., G.P. Fitzgerald, and R.H. Burris. 1968. Acetylene reduction by nitrogen-fixing blue-green algae. *Arch. Mikrobiol.* 62:336-348.
- Vanderhoef, L.N., C.-Y. Huang, R. Musil, and J. Williams. 1974. Nitrogen fixation (acetylene reduction) by phytoplankton in Green Bay, Lake Michigan, in relation to nutrient concentrations. *Limnol. Oceanogr.* 19:119-125.

TAB. 1. Some characteristic data for Lakes Trummen (0.2-1.5 m) and Södra Bergundasjön (1.0 m).

	L. Trummen 1973/74	L. S. Bergunda- sjön, 1975/76
Surface area, km ²	0.65	4.3
Lake volume, 10 ⁶ m ³	1.1	8.4
Maximum depth, m	2.5	5.4
Mean depth, m	1.8	2.3
Drainage area, km ²	13	44
Total N, mg N l ⁻¹	1.6/1.7 ^{1/}	2.3/3.3 ^{1/}
DIN, mg N l ⁻¹	0.06/0.02	0.23/0.46
Total P, mg P l ⁻¹	0.07/0.08	0.77/0.77
MRP, mg P l ⁻¹	0.01/0.01	0.47/0.48
Total Fe, mg l ⁻¹	0.8/0.9	0.8/0.5
Phytopl. biomass, mg FW l ⁻¹	8.3/10	16/27
Secchi depth, m	0.75/0.62 ^{2/}	0.55/0.47 ^{2/}

^{1/}For all concentrations volume-weighted means for the period June-September.

^{2/}Weighted mean for the period June-September.

TAB. 2. Supply of nitrogen from external and internal sources ($\text{mg N m}^{-2} 24 \text{ h}^{-1}$), and turnover rate of seston nitrogen (24 h^{-1}), in Lake Södra Bergundasjön during summer 1975 and 1976.

	1975							1976				
	27 May	18 Jun	15 Jul	6 Aug	27 Aug	16 Sep	2 Jun	15 Jul	9 Aug	5 Sep		
Phytoplankton N demand ^{1/}	77	110	180	160	78	0	160	240	160	150		
- N ₂ fixation	0	1	1	5	64	14	0	46	70	2		
- Import of DIN to lake ^{2/}	15	11	7	3	3	20	10	3	2	2		
+ Export of DIN from lake ^{2/}	7	5	1	0	0	18	3	2	0	0		
= Internally supplied	69	103	173	152	11	-16	153	193	88	146		
- Gross release from sed ^{3/}	41	39	82	162	121	52	40	80	160	90		
= Residual from seston	28	64	91	-10	-110	-68	113	113	-72	56		
Supply of N demand, %												
from sediment	60	40	50	110	860	∞	25	40	180	60		
from seston	40	60	50	0	0	0	75	60	0	40		
Seston conc., $\text{g N m}^{-2} 4/$	-	-	-	-	-	-	2.7	3.9	7.4	3.8		
Est. turnover rate of seston nitrogen, 24 h^{-1}	-	-	-	-	-	-	0.04	0.03	0.00	0.01		

1/ Calculated from net carbon uptake, assuming C:N ratio of 9.5:1, and respiration rate corresponding to 15% of P_{max} .

2/ Bengtsson (1978).

3/ Gross release from sediment estimated from laboratory nutrient-exchange experiments with "undisturbed" sediment cores (n=5) under anoxic conditions and at in-situ temperature during 1975 (L. Bengtsson pers. comm.); release rates during 1976 derived from 1975 study.

4/ Calculated from 0-1 m seston values, assuming even distribution in the 0-2 m surface layer.

LEGENDS

FIG. 1. Phytoplankton net carbon uptake, nitrogen fixation (solid line), volume-weighted mean concentration of dissolved inorganic nitrogen (dashed line), total phytoplankton biomass (solid line), and biomass of heterocystous blue-green algae (dashed line) in Lake Trummen. Net carbon uptake was calculated by subtracting respiration rates corresponding to 5% (5%R) and 20% (20%R) of $P_{\max}$ from daily carbon uptake. Sampling dates when nitrogen fixation supported nitrogen demands of heterocystous algae to 100% or more (*), and less than 100% (o) according to Fig. 4 are denoted.

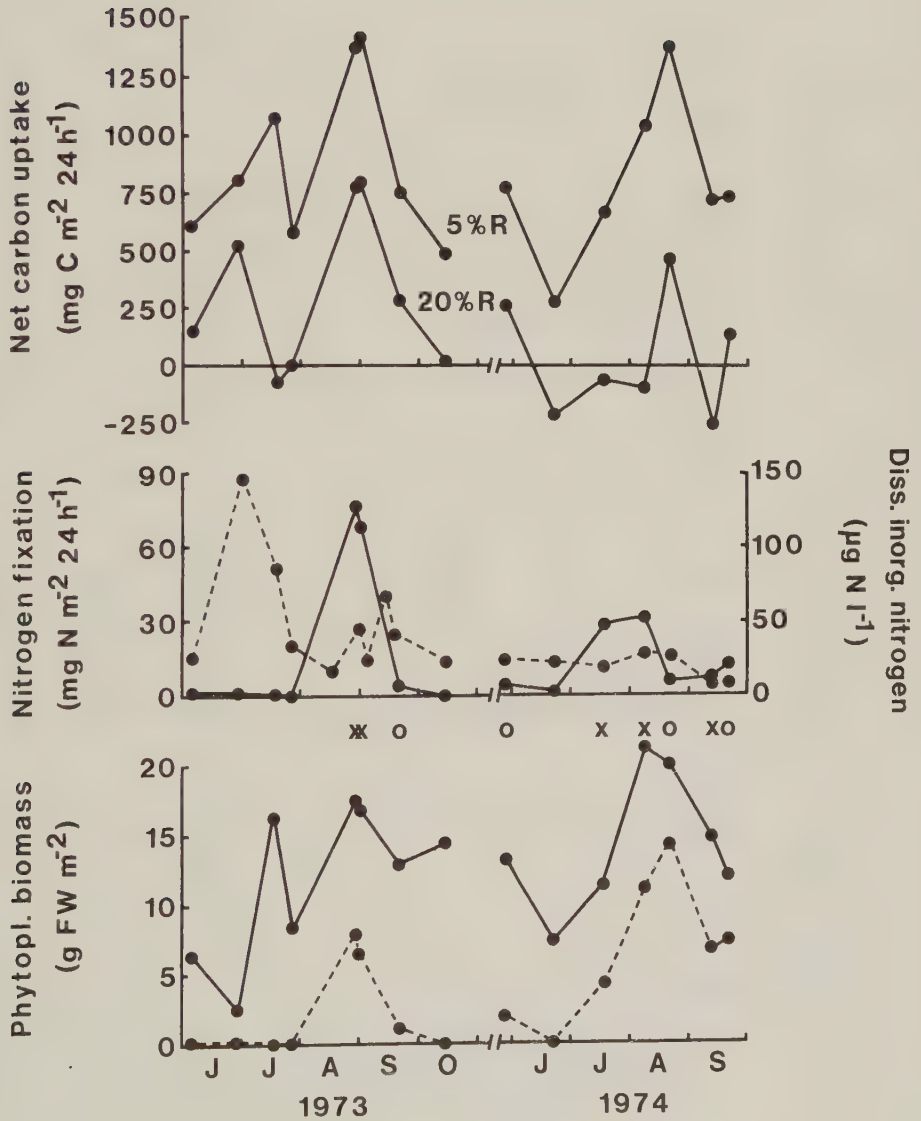
FIG. 2. Phytoplankton net carbon uptake, nitrogen fixation (solid line), volume-weighted mean concentration of dissolved inorganic nitrogen (dashed line), total phytoplankton biomass (solid line), and biomass of heterocystous blue-green algae (dashed line) in Lake Södra Bergundasjön. Net carbon uptake was calculated by subtracting respiration rates corresponding to 5% (5%R) and 20% (20%R) of $P_{\max}$ from daily carbon uptake. Sampling dates when nitrogen fixation supported nitrogen demands of heterocystous algae to 100% or more (*), and less than 100% (o) according to Fig. 4 are denoted.

FIG. 3. Seston C:N ratios (by weight) from epilimnion of Lakes Trummen and Södra Bergundasjön. Arithmetic mean and ranges (n=2).

FIG. 4. Percentage of total and heterocystous phytoplankton nitrogen requirements supplied by nitrogen fixation in Lakes Trummen (●) and Södra Bergundasjön (○). Heterocystous algae, expressed in % of total phytoplankton biomass, are assumed to have fulfilled their nitrogen requirements through N_2 fixation when data points lie on or above the dashed line (1:1). At 100% N supplied by N_2 fixation, the total phytoplankton demand was furnished. Diurnal nitrogen requirements estimated using carbon respiration rates corresponding to 15% of P_{max} , and phytoplankton C:N ratio of 9.5:1.

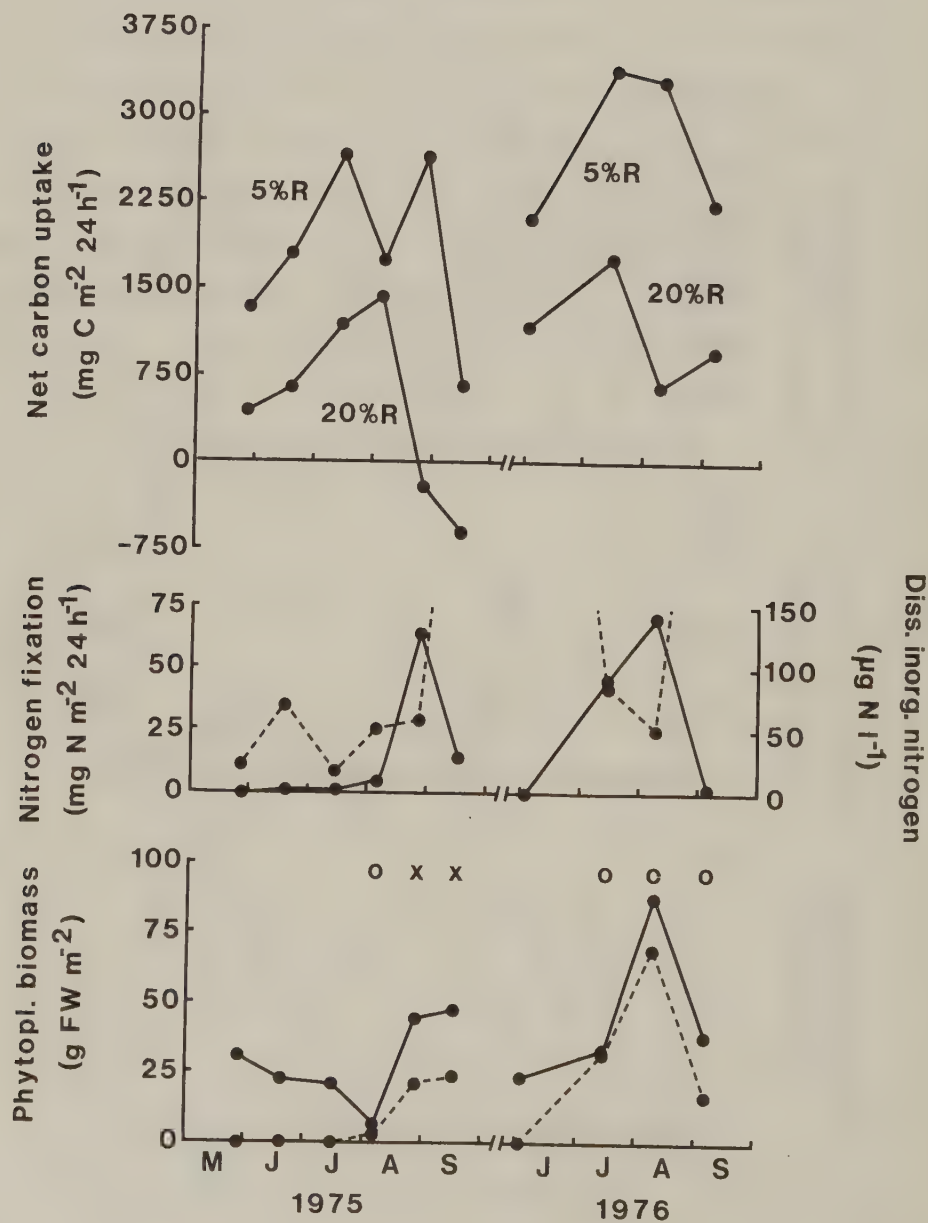
Lars Leonardson

FIG. 1.



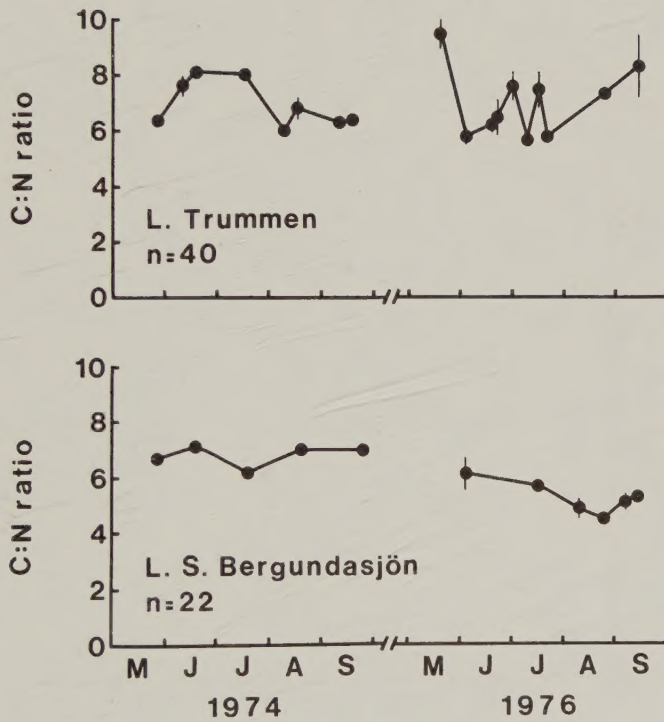
Lars Leonardson

FIG. 2.



Lars Leonardson

FIG. 3.



Lars Leonardson

FIG. 4.

